updated search L/Cook 4/26/04

incorporation; UGA codon position
IT Methods & Equipment
mutagenesis: analytical method, molecular genetics/genetic engineering; protein analysis: Analysis/Characterization Techniques: CB, analytical method
ORGN Classifier
Cercopithecidae 86205
Super Taxa
Primates; Mammalia; Vertebrata; Chordata; Animalia
Organism Name
COS-7: African green monkey kidney
Taxa Notes
Animals, Chordates, Mammals, Nonhuman Mammals, Nonhuman Vertebrates, Nonhuman Primates, Primates, Vertebrates
RN 3614-08-2 (selenocysteine)
70-18-8 (GLUTATHIONE)

=> d his

(FILE 'HOME' ENTERED AT 15:24:06 ON 26 APR 2004)

FILE 'BIOSIS, CAPLUS, EMBASE, MEDLINE, CANCERLIT, JAPIO' ENTERED AT 15:24:34 ON 26 APR 2004
L1 19 S GFP AND 157
L2 24 S GFP AND 172
L3 4 S L1 AND L2
L4 1 DUPLICATE REMOVE L3 (3 DUPLICATES REMOVED)
L5 6 DUPLICATE REMOVE L1 (13 DUPLICATES REMOVED)
L6 11 DUPLICATE REMOVE L2 (13 DUPLICATES REMOVED)

=>

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d his

     (FILE 'HOME' ENTERED AT 14:21:27 ON 26 APR 2004)

     FILE 'STNGUIDE' ENTERED AT 14:21:30 ON 26 APR 2004

     FILE 'HOME' ENTERED AT 14:21:34 ON 26 APR 2004

     FILE 'BIOSIS, CAPLUS, EMBASE, MEDLINE, CANCERLIT, JAPIO' ENTERED AT
     14:22:05 ON 26 APR 2004
L1           301 S (WILD TYPE GFP)
L2            54 S L1 AND SER?
L3            53 S L2 AND PRO?
L4             2 S L3 AND 147

=> s l1 and LEPRAS
L5             0 L1 AND LEPRAS

=>
Connection closed by remote host
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 d his

     (FILE 'HOME' ENTERED AT 14:21:27 ON 26 APR 2004)

     FILE 'STNGUIDE' ENTERED AT 14:21:30 ON 26 APR 2004

     FILE 'HOME' ENTERED AT 14:21:34 ON 26 APR 2004

     FILE 'BIOSIS, CAPLUS, EMBASE, MEDLINE, CANCERLIT, JAPIO' ENTERED AT
     14:22:05 ON 26 APR 2004
L1           301 S (WILD TYPE GFP)
L2            54 S L1 AND SER?
L3            53 S L2 AND PRO?
L4             2 S L3 AND 147

=> s l1 and LEPRAS
L5             0 L1 AND LEPRAS

=>
Connection closed by remote host
```

d l4 1 all

L4 ANSWER 1 OF 1 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
DUPLICATE 1
AN 2001:538663 BIOSIS
DN PREV200100538663
TI Green fluorescent protein rendered susceptible to proteolysis: Positions for protease-sensitive insertions.
AU Chiang, Cheng-Feng; Okou, David T.; Griffin, Tony B.; Verret, C. Reynold; Williams, Myron N. V. [Reprint author]
CS Department of Chemistry, Clark Atlanta University, Atlanta, GA, 30314, USA mnwill@cau.edu
SO Archives of Biochemistry and Biophysics, (October 15, 2001) Vol. 394, No. 2, pp. 229-235. print.
CODEN: ABBIA4. ISSN: 0003-9861.
DT Article
LA English
ED Entered STN: 21 Nov 2001
Last Updated on STN: 25 Feb 2002
AB The green fluorescent protein (**GFP**) is highly resistant to proteolysis and remains uncleaved after prolonged incubation with trypsin or pronase despite several putative tryptic and chymotryptic sites in exposed loops. We have rendered **GFP** sensitive to proteolysis by inserting five amino acids, IEGRS, in loops at position **157**, **172**, or 189. Excitation and emission maxima of the three insertion mutants were similar to those of wild type, but quantum yields of mutants OMEGA172 and OMEGA189 were lower, indicating increased freedom of the fluorophore. Trypsin cleaved the native (folded) form of each mutant at a unique site defined by the insert. Pronase also yields similar digestion patterns in these variants, but further proteolysis was also observed, suggesting that the primary cleavage relaxes **GFP** structure and reveals previously inaccessible sites. Fluorescence of OMEGA189 changed little upon digestion with trypsin but decreased progressively by as much as 40% upon digestion with increasing amounts of pronase. Fluorescence of other variants was not affected significantly by the proteases, further confirming the remarkable stabilities of **GFP** variants. These constructs define a new conformation-sensitive site around residue 189 of **GFP** and show that **GFP** may be useful for design of protease-susceptible molecules for monitoring of specific proteolytic activities in vivo.
CC Biochemistry studies - General 10060
Biochemistry studies - Proteins, peptides and amino acids 10064
Enzymes - General and comparative studies: coenzymes 10802
Physiology and biochemistry of bacteria 31000
IT Major Concepts
Biochemistry and Molecular Biophysics
IT Chemicals & Biochemicals
factor Xa; green fluorescent protein; pronase; protease; trypsin
IT Methods & Equipment
mutagenesis: analytical method, genetic engineering, genetic method
IT Miscellaneous Descriptors
protease-sensitive insertions; proteolysis
ORGN Classifier
Bacteria 05000
Super Taxa
Microorganisms
Organism Name
bacteria
Taxa Notes
Bacteria, Eubacteria, Microorganisms
RN 9002-05-5 (factor Xa)
9036-06-0 (pronase)

9001-92-7 (protease)
9002-07-7 (trypsin)

=> d his

(FILE 'HOME' ENTERED AT 15:24:06 ON 26 APR 2004)

FILE 'BIOSIS, CAPLUS, EMBASE, MEDLINE, CANCERLIT, JAPIO' ENTERED AT 15:24:34 ON 26 APR 2004
L1 19 S GFP AND 157
L2 24 S GFP AND 172
L3 4 S L1 AND L2
L4 1 DUPLICATE REMOVE L3 (3 DUPLICATES REMOVED)

=> duplicate remove l1
DUPLICATE PREFERENCE IS 'BIOSIS, CAPLUS, EMBASE, MEDLINE, CANCERLIT'
KEEP DUPLICATES FROM MORE THAN ONE FILE? Y/(N):n
PROCESSING COMPLETED FOR L1
L5 6 DUPLICATE REMOVE L1 (13 DUPLICATES REMOVED)

=> d l5 1-6 all

L5 ANSWER 1 OF 6 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
DUPLICATE 1
AN 2003:261418 BIOSIS
DN PREV200300261418
TI The sigmaE regulon and the identification of additional sporulation genes in Bacillus subtilis.
AU Eichenberger, Patrick; Jensen, Shane T.; Conlon, Erin M.; van Ooij, Christiaan; Silvaggi, Jessica; Gonzalez-Pastor, Jose-Eduardo; Fujita, Masaya; Ben-Yehuda, Sigal; Stragier, Patrick; Liu, Jun S.; Losick, Richard [Reprint Author]
CS Department of Molecular and Cellular Biology, Harvard University Biological Laboratories, 16 Divinity Avenue, Cambridge, MA, 02138, USA losick@mcb.harvard.edu
SO Journal of Molecular Biology, (11 April 2003) Vol. 327, No. 5, pp. 945-972. print.
ISSN: 0022-2836 (ISSN print).
DT Article
LA English
ED Entered STN: 4 Jun 2003
Last Updated on STN: 4 Jun 2003
AB We report the identification and characterization on a genome-wide basis of genes under the control of the developmental transcription factor sigmaE in Bacillus subtilis. The sigmaE factor governs gene expression in the larger of the two cellular compartments (the mother cell) created by polar division during the developmental process of sporulation. Using transcriptional profiling and bioinformatics we show that 253 genes (organized in **157** operons) appear to be controlled by sigmaE. Among these, 181 genes (organized in 121 operons) had not been previously described as members of this regulon. Promoters for many of the newly identified genes were located by transcription start site mapping. To assess the role of these genes in sporulation, we created null mutations in 98 of the newly identified genes and operons. Of the resulting mutants, 12 (in prkA, ybaN, yhbH, ykvV, ylbJ, ypjB, yqfC, yqfD, ytrH, ytrI, ytvI and yunB) exhibited defects in spore formation. In addition, subcellular localization studies were carried out using in-frame fusions of several of the genes to the coding sequence for **GFP**. A majority of the fusion proteins localized either to the membrane surrounding the developing spore or to specific layers of the spore coat, although some fusions showed a uniform distribution in the mother cell

cytoplasm. Finally, we used comparative genomics to determine that 46 of the sigmaE-controlled genes in B. subtilis were present in all of the Gram-positive endospore-forming bacteria whose genome has been sequenced, but absent from the genome of the closely related but not endospore-forming bacterium Listeria monocytogenes, thereby defining a core of conserved sporulation genes of probable common ancestral origin. Our findings set the stage for a comprehensive understanding of the contribution of a cell-specific transcription factor to development and morphogenesis.

CC Cytology - General 02502
Genetics - General 03502
Biochemistry studies - Nucleic acids, purines and pyrimidines 10062
Development and Embryology - General and descriptive 25502
Morphology and cytology of bacteria 30500
Physiology and biochemistry of bacteria 31000
Genetics of bacteria and viruses 31500

IT Major Concepts
Cell Biology; Development; Molecular Genetics (Biochemistry and Molecular Biophysics)

IT Chemicals & Biochemicals
sigma factors; sigma-E: developmental transcription factor; sporulation genes: identification

IT Methods & Equipment
cytology: histology and cytology techniques, laboratory techniques

IT Miscellaneous Descriptors
bacterial genomics; bioinformatics; genome; polar division; protein localization; sigma-E regulation; sporulation

ORGN Classifier
Endospore-forming Gram-Positives 07810
Super Taxa
Eubacteria; Bacteria; Microorganisms
Organism Name
Bacillus subtilis (species): sporulation
Taxa Notes
Bacteria, Eubacteria, Microorganisms

ORGN Classifier
Regular Nonsporing Gram-Positive Rods 07830
Super Taxa
Eubacteria; Bacteria; Microorganisms
Organism Name
Listeria monocytogenes (species)
Taxa Notes
Bacteria, Eubacteria, Microorganisms

L5 ANSWER 2 OF 6 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
AN 2004:180837 BIOSIS
DN PREV200400180886
TI Hereditary spherocytosis mutations in an erythroid ankyrin insulator element are associated with gene silencing in vitro and promoter dysfunction in transgenic mice.
AU Liem, Robert I. [Reprint Author]; Seidel, Nancy E. [Reprint Author]; Wong, Clara; Cline, Amanda P. [Reprint Author]; Gallagher, Patrick G.; Bodine, David M. [Reprint Author]
CS Hematopoiesis Section, NHGRI, Bethesda, MD, USA
SO Blood, (November 16 2003) Vol. 102, No. 11, pp. 7a. print.
Meeting Info.: 45th Annual Meeting of the American Society of Hematology. San Diego, CA, USA. December 06-09, 2003. American Society of Hematology.
CODEN: BLOOAW. ISSN: 0006-4971.
DT Conference; (Meeting)
Conference; Abstract; (Meeting Abstract)
LA English
ED Entered STN: 7 Apr 2004

Last Updated on STN: 7 Apr 2004

AB Mutations at positions -108 and -153 of the human erythroid ankyrin-1 (ANK-1) promoter are associated with non-dominant hereditary spherocytosis, resulting in hemolytic anemia. However, the mechanism by which the mutations cause ankyrin deficiency is unknown. Using a transgenic mouse assay, we have shown that the ANK-1 promoter containing the -108/-153 mutations fused to the human gamma-globin gene resulted in low-level, position dependent, copy number independent and variegated expression of the transgene (17 lines) when compared to 32 lines containing the wild type (WT) promoter. Normal function of the -108/-153 promoter was restored by flanking the transgene with the insulator from the chicken beta-globin locus (5'HS4-chHS4). Insulators are associated with DNase I hypersensitive sites (HS) at the boundary between chromatin that is resistant to DNase I digestion and DNase I sensitive chromatin. Insulators are defined by a "barrier" function, which prevents gene silencing, and may also have enhancer blocking activity. We hypothesized that the WT ANK-1 promoter contains an insulator that is disrupted by the -108/-153 mutations. To test this hypothesis, we digested nuclei from K562 cells with increasing amounts of DNase I and demonstrated a 200 bp HS over the erythroid ANK-1 promoter between -98 and -296. No HS were detected in a 10 kb region of DNase I resistant chromatin upstream of the ANK-1 promoter, and DNase I sensitive chromatin extended at least 2 kb downstream, demonstrating that the HS is at the boundary between DNase I resistant and sensitive chromatin. Using chromatin immunoprecipitation (ChIP), we demonstrated that GATA-1 and CBP occupied the region between -**157** and +69 of the WT ANK-1 promoter and that histones H3 and H4 were hyperacetylated in this region. Gel shift assays with the WT promoter showed that Brg-1, which provides the ATPase activity of the SWI/SNF chromatin remodeling complex and is associated with barrier function in chHS4, and CTCF, which is required for enhancer blocking in chHS4, bound to the sequence between -99 and -159, but not to the same sequence with the -108/-153 mutations. DNase I treatment of fetal liver cells from transgenic mice revealed the ANK-1 HS was present in animals containing the WT promoter, but HS formation was inhibited by the -108/-153 mutations. We tested the sequence between -98 and -296 from both the WT and the -108/-153 ANK-1 promoter for its ability to suppress gene silencing in vitro. FACS analysis of K562 cells transfected with a construct containing HS2 from the human beta-globin locus and the gamma-globin promoter linked to the **GFP** gene showed rapid silencing of the transgene in 8/8 cell lines. Gene silencing was not observed in cell lines transfected with either 2 (12 cell lines) or 4 copies (12 cell lines) of the WT ANK-1 sequence flanking the transgene. Gene silencing was observed in 5/10 cell lines containing 2 copies of the -108/-153 ANK-1 sequence flanking the transgene (chi2=14.95, p<y0.001). Similar to the insulator at the 3' end of the chicken beta-globin locus, the WT ANK-1 sequence did not block the effects of the HS2 enhancer in K562 cells. We conclude that the erythroid ANK-1 promoter contains an insulator that is associated with a DNase I HS and that the -108/-153 mutations disturb binding of critical chromatin remodeling proteins, leading to position effects in transgenic mice and gene silencing in K562 cells. We propose the -108/-153 mutations may lead to ankyrin deficient erythrocytes in spherocytosis patients by a similar mechanism.

CC General biology - Symposia, transactions and proceedings 00520
Genetics - General 03502
Genetics - Animal 03506
Genetics - Human 03508
Blood - Blood and lymph studies 15002
Blood - Blood cell studies 15004
Blood - Blood, lymphatic and reticuloendothelial pathologies 15006

IT Major Concepts
Blood and Lymphatics (Transport and Circulation); Molecular Genetics (Biochemistry and Molecular Biophysics)

IT Parts, Structures, & Systems of Organisms
blood: blood and lymphatics; erythroid: blood and lymphatics
IT Diseases
hereditary spherocytosis: blood and lymphatic disease, genetic disease, genetics
Spherocytosis, Hereditary (MeSH)
IT Methods & Equipment
gene silencing: genetic techniques, laboratory techniques
ORGN Classifier
Hominidae 86215
Super Taxa
Primates; Mammalia; Vertebrata; Chordata; Animalia
Organism Name
K562 cell line (cell line): human chronic myelogenous leukemia cells
human (common)
Taxa Notes
Animals, Chordates, Humans, Mammals, Primates, Vertebrates
ORGN Classifier
Muridae 86375
Super Taxa
Rodentia; Mammalia; Vertebrata; Chordata; Animalia
Organism Name
mouse (common): transgenic
Taxa Notes
Animals, Chordates, Mammals, Nonhuman Vertebrates, Nonhuman Mammals, Rodents, Vertebrates
GEN human ANK-1 gene [human ankyrin-1 gene] (Hominidae): mutation, promoter region; human alpha-globin gene (Hominidae): promoter region; human beta-globin gene (Hominidae): locus

L5 ANSWER 3 OF 6 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
DUPLICATE 2
AN 2002:337041 BIOSIS
DN PREV200200337041
TI Transduction of interphase cells by avian sarcoma virus.
AU Katz, Richard A. [Reprint author]; Greger, James G.; Darby, Kristen; Boimel, Pamela; Rall, Glenn F.; Skalka, Anna Marie
CS Fox Chase Cancer Center, Institute for Cancer Research, 7701 Burholme Ave., Philadelphia, PA, 19111, USA
R_Katz@fccc.edu
SO Journal of Virology, (June, 2002) Vol. 76, No. 11, pp. 5422-5434. print.
CODEN: JOVIAM. ISSN: 0022-538X.
DT Article
LA English
ED Entered STN: 12 Jun 2002
Last Updated on STN: 12 Jun 2002
AB It has been generally believed that oncoretroviruses are dependent on mitosis for efficient nuclear entry of viral DNA. We previously identified a nuclear localization signal in the integrase protein of an oncoretrovirus, avian sarcoma virus (ASV), suggesting an active import mechanism for the integrase-DNA complex (G. Kukolj, R. A. Katz, and A. M. Skalka, Gene 223:**157**-163, 1998). Here, we have evaluated the requirement for mitosis in nuclear import and integration of ASV DNA. Using a modified ASV encoding a murine leukemia virus amphotropic env gene and a green fluorescent protein (**GFP**) reporter gene, DNA nuclear import was measured in cell cycle-arrested avian (DF-1) as well as human (HeLa) and mouse cells. The results showed efficient accumulation of nuclear forms of ASV DNA in gamma-irradiation-arrested cells. Efficient transduction of a **GFP** reporter gene was also observed after infection of cells that were arrested with gamma-irradiation, mitomycin C, nocodazole, or aphidicolin, confirming that nuclear import and integration of ASV DNA can occur in the absence of mitosis. By monitoring **GFP**

expression in individual cells, we also obtained evidence for nuclear import of viral DNA during interphase in cycling cells. Lastly, we observed that ASV can transduce postmitotic mouse neurons. These results support an active nuclear import mechanism for the oncoretrovirus ASV and suggest that this mechanism can operate in both nondividing and dividing cells.

CC Cytology - General 02502
Cytology - Animal 02506
Cytology - Human 02508
Biochemistry studies - General 10060
Biochemistry studies - Nucleic acids, purines and pyrimidines 10062
Biochemistry studies - Proteins, peptides and amino acids 10064
Enzymes - General and comparative studies: coenzymes 10802
Nervous system - Physiology and biochemistry 20504
Genetics of bacteria and viruses 31500
Virology - Animal host viruses 33506
Medical and clinical microbiology - Virology 36006

IT Major Concepts
Biochemistry and Molecular Biophysics; Cell Biology; Infection

IT Parts, Structures, & Systems of Organisms
neurons: nervous system

IT Chemicals & Biochemicals
DNA; enzymes; green fluorescent protein reporter genes; integrases; proteins; viral DNA: nuclear entry mechanisms

IT Miscellaneous Descriptors
cell division; interphase cells: viral transduction; mitosis; viral genomes: integration

ORGN Classifier
Aves 85500
Super Taxa
Vertebrata; Chordata; Animalia
Organism Name
bird: host
Taxa Notes
Animals, Birds, Chordates, Nonhuman Vertebrates, Vertebrates

ORGN Classifier
Hominidae 86215
Super Taxa
Primates; Mammalia; Vertebrata; Chordata; Animalia
Organism Name
HeLa cell line
human
Taxa Notes
Animals, Chordates, Humans, Mammals, Primates, Vertebrates

ORGN Classifier
Muridae 86375
Super Taxa
Rodentia; Mammalia; Vertebrata; Chordata; Animalia
Organism Name
mouse
Taxa Notes
Animals, Chordates, Mammals, Nonhuman Vertebrates, Nonhuman Mammals, Rodents, Vertebrates

ORGN Classifier
Retroviridae 03305
Super Taxa
DNA and RNA Reverse Transcribing Viruses; Viruses; Microorganisms
Organism Name
avian sarcoma virus: pathogen
retrovirus: pathogen
Taxa Notes
DNA and RNA Reverse Transcribing Viruses, Microorganisms, Viruses

RN 52350-85-3 (integrases)

L5 ANSWER 4 OF 6 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
DUPLICATE 3
AN 2001:538663 BIOSIS
DN PREV200100538663
TI Green fluorescent protein rendered susceptible to proteolysis: Positions for protease-sensitive insertions.
AU Chiang, Cheng-Feng; Okou, David T.; Griffin, Tony B.; Verret, C. Reynold; Williams, Myron N. V. [Reprint author]
CS Department of Chemistry, Clark Atlanta University, Atlanta, GA, 30314, USA
mnwill@cau.edu
SO Archives of Biochemistry and Biophysics, (October 15, 2001) Vol. 394, No. 2, pp. 229-235. print.
CODEN: ABBIA4. ISSN: 0003-9861.
DT Article
LA English
ED Entered STN: 21 Nov 2001
Last Updated on STN: 25 Feb 2002
AB The green fluorescent protein (**GFP**) is highly resistant to proteolysis and remains uncleaved after prolonged incubation with trypsin or pronase despite several putative tryptic and chymotryptic sites in exposed loops. We have rendered **GFP** sensitive to proteolysis by inserting five amino acids, IEGRS, in loops at position **157**, 172, or 189. Excitation and emission maxima of the three insertion mutants were similar to those of wild type, but quantum yields of mutants OMEGA172 and OMEGA189 were lower, indicating increased freedom of the fluorophore. Trypsin cleaved the native (folded) form of each mutant at a unique site defined by the insert. Pronase also yields similar digestion patterns in these variants, but further proteolysis was also observed, suggesting that the primary cleavage relaxes **GFP** structure and reveals previously inaccessible sites. Fluorescence of OMEGA189 changed little upon digestion with trypsin but decreased progressively by as much as 40% upon digestion with increasing amounts of pronase. Fluorescence of other variants was not affected significantly by the proteases, further confirming the remarkable stabilities of **GFP** variants. These constructs define a new conformation-sensitive site around residue 189 of **GFP** and show that **GFP** may be useful for design of protease-susceptible molecules for monitoring of specific proteolytic activities in vivo.
CC Biochemistry studies - General 10060
Biochemistry studies - Proteins, peptides and amino acids 10064
Enzymes - General and comparative studies: coenzymes 10802
Physiology and biochemistry of bacteria 31000
IT Major Concepts
Biochemistry and Molecular Biophysics
IT Chemicals & Biochemicals
factor Xa; green fluorescent protein; pronase; protease; trypsin
IT Methods & Equipment
mutagenesis: analytical method, genetic engineering, genetic method
IT Miscellaneous Descriptors
protease-sensitive insertions; proteolysis
ORGN Classifier
Bacteria 05000
Super Taxa
Microorganisms
Organism Name
bacteria
Taxa Notes
Bacteria, Eubacteria, Microorganisms
RN 9002-05-5 (factor Xa)
9036-06-0 (pronase)

9001-92-7 (protease)
9002-07-7 (trypsin)

L5 ANSWER 5 OF 6 CAPLUS COPYRIGHT 2004 ACS on STN
AN 2000:359911 CAPLUS
DN 133:164324
ED Entered STN: 31 May 2000
TI Antiparallel Leucine Zipper-Directed Protein Reassembly: Application to the Green Fluorescent Protein
AU Ghosh, Indraneel; Hamilton, Andrew D.; Regan, Lynne
CS Department of Chemistry, Yale University, New Haven, CT, 06520, USA
SO Journal of the American Chemical Society (2000), 122(23), 5658-5659
CODEN: JACSAT; ISSN: 0002-7863
PB American Chemical Society
DT Journal
LA English
CC 34-4 (Amino Acids, Peptides, and Proteins)
Section cross-reference(s): 6
AB A general method for reassembly of protein fragments mediated by noncovalent association of antiparallel leucine zippers was described, using green fluorescent protein (**GFP**) from Aequorea victoria as an example. **GFP** was dissected between residues **157**-158, a position that has been shown to accommodate a 20-residue amino acid insertion; the fragments were termed NGFP (N-terminal-containing) and CGFP(C-terminal-containing), resp. Two designed helixes, NZ or CZ, were attached, NZ to the NGFP C-terminal by a six-residue linker, and CZ to the CGFP N-terminal by a four-residue linker, to generate fragments NZGFP and CZGFP, resp. Genes coding for NZGFP and CZGFP were cloned and expressed using standard methods, and the proteins isolated. Equimolar amts. of the NZGFP and CZGFP fragments were denatured and dialyzed, and the reassembled peptides were visibly green and had fluorescence excitations and emission spectra identical to that of parent **GFP**.
ST antiparallel leucine zipper reassembly green fluorescent protein
IT Fluorescence
Protein folding
Self-assembly
(antiparallel leucine zipper-directed protein reassembly of green fluorescent protein)
IT Peptides, properties
Proteins, general, properties
RL: PRP (Properties)
(antiparallel leucine zipper-directed protein reassembly of green fluorescent protein)
IT 288154-44-9P 288154-45-0P 288154-47-2P 288154-48-3P 288154-49-4P
RL: BPN (Biosynthetic preparation); PRP (Properties); SPN (Synthetic preparation); BIOL (Biological study); PREP (Preparation)
(antiparallel leucine zipper-directed protein reassembly of green fluorescent protein)
RE.CNT 39 THERE ARE 39 CITED REFERENCES AVAILABLE FOR THIS RECORD
RE
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L5 ANSWER 6 OF 6 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
DUPLICATE 4
AN 2000:533558 BIOSIS
DN PREV200000533558
TI Gene structure and expression study of the SEDL gene for spondyloepiphyseal dysplasia tarda.
AU Gecz, Jozef [Reprint author]; Hillman, Marie A.; Gedeon, Agi K.; Cox, Timothy C.; Baker, Elizabeth; Mulley, John C.
CS Department of Cytogenetics and Molecular Genetics, Women's and Children's Hospital, 72 King William Road, North Adelaide, SA, 5006: jgecz@medicine.adelaide.edu.au, Australia
SO Genomics, (October 15, 2000) Vol. 69, No. 2, pp. 242-251. print.
CODEN: GNMCEP. ISSN: 0888-7543.
DT Article
LA English
ED Entered STN: 13 Dec 2000
Last Updated on STN: 11 Jan 2002
AB Spondyloepiphyseal dysplasia tarda (SEDL) is an X-linked recessive disorder of endochondral bone formation caused by mutations in the SEDL gene. Here we present the structural analysis and subcellular localization of human SEDL. The SEDL gene is composed of six exons and spans a genomic region of apprx20 kb in Xp22. It contains four Alu sequences in its 3' UTR and an alternatively spliced MER20 sequence in its 5' UTR (exon 2). Complex alternative splicing was detected for exon 4. Altogether seven SEDL pseudogenes were detected in the human genome: SEDLP1, a transcribed retropseudogene (or retro-xaptonuon) on chromosome 19q13.4 with potential to encode a protein identical to that of the SEDL gene; SEDLP2, another retropseudogene (not transcribed) on chromosome 8; and five truncated pseudogenes, SEDLP3-SEDLP7, on chromosome Yq11.23. Based on the knowledge of the yeast SEDL ortholog we speculated that the SEDL protein may participate along the ER-to-Golgi transport compartments. To test this hypothesis we performed transient transfection studies with tagged recombinant mammalian SEDL proteins in Cos-7 cells. The tagged

SEDL proteins localized to perinuclear structures that partly overlapped with the intermediate ER-Golgi compartment (ERGIC; or vesicular tubular complex, VTC). Two human SEDL mutations (**157**-158delAT and C271T(STOP)) introduced into SEDL FLAG and **GFP** constructs led to the misplacement of the SEDL protein primarily to the cell nucleus and partially to the cytoplasm. Based on these experiments we suggest that the COOH end of the SEDL protein might be responsible for proper targeting of SEDL along the ER-Golgi membrane compartments (including Golgi and ERGIC/VTC).

CC Bones, joints, fasciae, connective and adipose tissue - Pathology 18006
Cytology - Animal 02506
Cytology - Human 02508
Genetics - General 03502
Genetics - Animal 03506
Genetics - Human 03508
Bones, joints, fasciae, connective and adipose tissue - Physiology and biochemistry 18004
Development and Embryology - Pathology 25503

IT Major Concepts
Molecular Genetics (Biochemistry and Molecular Biophysics); Skeletal System (Movement and Support)

IT Parts, Structures, & Systems of Organisms
X chromosome

IT Diseases
spondyloepiphyseal dysplasia tarda: bone disease, congenital disease, X-linked recessive disorder

IT Miscellaneous Descriptors
human genome

ORGN Classifier
Cercopithecidae 86205
Super Taxa
Primates; Mammalia; Vertebrata; Chordata; Animalia
Organism Name
Cos-7 cell line
Taxa Notes
Animals, Chordates, Mammals, Nonhuman Mammals, Nonhuman Vertebrates, Nonhuman Primates, Primates, Vertebrates

GEN human SEDL gene (Hominidae): expression study, gene structure, subcellular localization; human SEDLP1 pseudogene (Hominidae): expression study, gene structure, subcellular localization

=> d his

(FILE 'HOME' ENTERED AT 15:24:06 ON 26 APR 2004)

FILE 'BIOSIS, CAPLUS, EMBASE, MEDLINE, CANCERLIT, JAPIO' ENTERED AT 15:24:34 ON 26 APR 2004

L1 19 S GFP AND 157
L2 24 S GFP AND 172
L3 4 S L1 AND L2
L4 1 DUPLICATE REMOVE L3 (3 DUPLICATES REMOVED)
L5 6 DUPLICATE REMOVE L1 (13 DUPLICATES REMOVED)

=> duplicate remove l2
DUPLICATE PREFERENCE IS 'BIOSIS, CAPLUS, EMBASE, MEDLINE, CANCERLIT'
KEEP DUPLICATES FROM MORE THAN ONE FILE? Y/(N):n
PROCESSING COMPLETED FOR L2
L6 11 DUPLICATE REMOVE L2 (13 DUPLICATES REMOVED)

=> d l6 1-11 all

L6 ANSWER 1 OF 11 CAPLUS COPYRIGHT 2004 ACS on STN
AN 2003:855957 CAPLUS
DN 139:347345
ED Entered STN: 31 Oct 2003
TI Functional complementation of two green fluorescent protein fragments and their use in a method for detecting protein - protein interactions and drug screening
IN Dahl, Soren Weis; Terry, Bernard Robert
PA Bioimage A/S, Den.
SO PCT Int. Appl., 96 pp.
CODEN: PIXXD2
DT Patent
LA English
IC ICM C07K014-435
ICS G01N033-68
CC 6-3 (General Biochemistry)
Section cross-reference(s): 1, 3, 9
FAN.CNT 2

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 2003089464	A1	20031030	WO 2002-DK882	20021219
	W: AE, AG, AL, AM, AT, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, CZ, DE, DE, DK, DK, DM, DZ, EC, EE, EE, ES, FI, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SK, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ				
	RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG				
	US 2003219717	A1	20031127	US 2003-370570	20030224
	WO 2003089627	A1	20031030	WO 2003-DK266	20030422
	W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, CZ, DE, DE, DK, DK, DM, DZ, EC, EE, EE, ES, FI, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG				
	RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG				
PRAI	DK 2002-591	A	20020419		
	DK 2002-896	A	20020613		
	DK 2002-1029	A	20020701		
	WO 2002-DK882	A1	20021219		

AB Fluorescence complementation products with intensity levels mimicking the full length intensities are obtained by introduction of improved folding capabilities with a mutation in position preceding the chromophore. The present application discloses that certain Green Fluorescent Proteins (**GFPs**) can be reassembled and form a functional fluorescent protein when expressed as two independent proteins halves. For example, when EGFP is expressed in mammalian cells, choosing a split site located in a loop region between the residues that form the beta-sheet structures of the **GFP** beta-barrel results in intense fluorescence. An additive increase is obtained by splitting the **GFP** between amino acids **172** and 173. The present application further illustrates that yellow variant of **GFP**, EYFP, is also reassembled and, surprisingly, the fluorescence from the reassembled protein is markedly

enhanced if it contains the F64L mutation. The reassembly of proteins does not occur if the two independent proteins halves are fused to non-interacting proteins. But, when brought together, they are reassembled to form a functional fluorescent protein. Screening for drugs capable of preventing interaction between proteins is performed by selecting the cells with the highest dynamic range through Fluorescence Activated Cell Sorting (FACS), as illustrated with the ability of FK506 to break the rapamycin induced interaction between FRB and FKBP.

ST green fluorescent protein fragment functional complementation protein interaction detection; **GFP** fragment functional complementation protein interaction detection drug screening

IT Cytometry
(FACS (fluorescence-activated cell sorting); functional complementation of two green fluorescent protein fragments and their use in method for detecting protein - protein interactions and drug screening)

IT Renilla
(**GFP** from; functional complementation of two green fluorescent protein fragments and their use in method for detecting protein - protein interactions and drug screening)

IT Proteins
RL: ARU (Analytical role, unclassified); BUU (Biological use, unclassified); ANST (Analytical study); BIOL (Biological study); USES (Uses)
(cyan fluorescent, enhanced, ECFP; functional complementation of two green fluorescent protein fragments and their use in method for detecting protein - protein interactions and drug screening)

IT Plasmid vectors
(encoding N- or C-terminal half of **GFP**; functional complementation of two green fluorescent protein fragments and their use in method for detecting protein - protein interactions and drug screening)

IT Protein folding
(fluorescent, improved capabilities; functional complementation of two green fluorescent protein fragments and their use in method for detecting protein - protein interactions and drug screening)

IT Molecular cloning
(functional expression; functional complementation of two green fluorescent protein fragments and their use in method for detecting protein - protein interactions and drug screening)

IT Proteins
RL: ARU (Analytical role, unclassified); BUU (Biological use, unclassified); ANST (Analytical study); BIOL (Biological study); USES (Uses)
(green fluorescent, **GFP**, mutated; functional complementation of two green fluorescent protein fragments and their use in method for detecting protein - protein interactions and drug screening)

IT Proteins
RL: ARU (Analytical role, unclassified); BUU (Biological use, unclassified); ANST (Analytical study); BIOL (Biological study); USES (Uses)
(green fluorescent, enhanced, EGFP; functional complementation of two green fluorescent protein fragments and their use in method for detecting protein - protein interactions and drug screening)

IT Proteins
RL: ANT (Analyte); BSU (Biological study, unclassified); ANST (Analytical study); BIOL (Biological study)
(interaction detection; functional complementation of two green fluorescent protein fragments and their use in method for detecting protein - protein interactions and drug screening)

IT Conformation
(loop, protein, of **GFP**, split site located in; functional complementation of two green fluorescent protein fragments and their

use in method for detecting protein - protein interactions and drug screening)

IT Animal cell
(mammalian, protein - protein interactions in; functional complementation of two green fluorescent protein fragments and their use in method for detecting protein - protein interactions and drug screening)

IT Chromophores
(mutated amino acid preceding; functional complementation of two green fluorescent protein fragments and their use in method for detecting protein - protein interactions and drug screening)

IT Drug screening
(of modulators of protein-protein interaction; functional complementation of two green fluorescent protein fragments and their use in method for detecting protein - protein interactions and drug screening)

IT Proteins
RL: ARU (Analytical role, unclassified); BUU (Biological use, unclassified); ANST (Analytical study); BIOL (Biological study); USES (Uses)
(red fluorescent, DsRed; functional complementation of two green fluorescent protein fragments and their use in method for detecting protein - protein interactions and drug screening)

IT Animal cell line
(stably expressing heterologous conjugate comprising protein of interest, establishing of; functional complementation of two green fluorescent protein fragments and their use in method for detecting protein - protein interactions)

IT Proteins
RL: ARU (Analytical role, unclassified); BUU (Biological use, unclassified); ANST (Analytical study); BIOL (Biological study); USES (Uses)
(yellow fluorescent, enhanced, EYFP; functional complementation of two green fluorescent protein fragments and their use in method for detecting protein - protein interactions and drug screening)

IT 618126-07-1
RL: PRP (Properties)
(Unclaimed; functional complementation of two green fluorescent protein fragments and their use in a method for detecting protein - protein interactions and drug screening)

IT 618126-03-7, 7: PN: WO03089464 SEQID: 7 unclaimed DNA 618126-05-9, 9: PN: WO03089464 SEQID: 9 unclaimed DNA 618126-08-2 618126-09-3
618126-10-6 618126-11-7 618126-12-8 618126-13-9 618126-14-0
618126-15-1 618126-16-2 618126-17-3 618126-18-4 618126-19-5
618126-20-8 618126-21-9 618126-22-0 618126-23-1 618126-24-2
618126-25-3 618126-26-4 618126-27-5 618126-28-6 618126-29-7
618126-30-0 618126-31-1 618126-32-2 618126-33-3 618126-34-4
618126-35-5 618126-36-6 618126-37-7 618126-38-8 618126-39-9
618126-40-2 618126-41-3 618126-42-4 618126-43-5
RL: PRP (Properties)
(unclaimed nucleotide sequence; functional complementation of two green fluorescent protein fragments and their use in a method for detecting protein - protein interactions and drug screening)

IT 618125-97-6 618125-98-7 618125-99-8 618126-00-4 618126-01-5
618126-02-6 618126-04-8 618126-06-0
RL: PRP (Properties)
(unclaimed protein sequence; functional complementation of two green fluorescent protein fragments and their use in a method for detecting protein - protein interactions and drug screening)

IT 618070-80-7 618070-81-8
RL: PRP (Properties)
(unclaimed sequence; functional complementation of two green

fluorescent protein fragments and their use in a method for detecting protein - protein interactions and drug screening)

RE.CNT 7 THERE ARE 7 CITED REFERENCES AVAILABLE FOR THIS RECORD

RE

(1) Hamilton, A; WO 0187919 A 2001 CAPLUS
(2) Japan Science & Tech Corp; EP 1229330 A 2002 CAPLUS
(3) Nagai, T; PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA 2001, V98(6), P3197 CAPLUS
(4) Ozawa, T; ANALYTICAL CHEMISTRY 2000, V72(21), P5151 CAPLUS
(5) Ozawa, T; ANALYTICAL CHEMISTRY 2001, V73(24), P5866 CAPLUS
(6) Palm, G; NATURE STRUCTURAL BIOLOGY 1997, V4(5), P361 CAPLUS
(7) Stratagene Inc; WO 0164843 A 2001 CAPLUS

L6 ANSWER 2 OF 11 CAPLUS COPYRIGHT 2004 ACS on STN
AN 2003:203300 CAPLUS
DN 138:232974
ED Entered STN: 14 Mar 2003
TI Method for production of mutant library of protein with various sizes and sequences using PCR-coupled recombination or sequence-directed recombination
IN Kim, Hak Sung; Kim, Geun Joong; Cheon, Young Hoon; Lee, Dong Eun
PA Korea Advanced Institute of Science and Technology, S. Korea
SO U.S. Pat. Appl. Publ., 12 pp.
CODEN: USXXCO
DT Patent
LA English
IC C12Q001-68; G01N033-53; C12P019-34; C12P021-02; C12N005-06; C07K014-00
NCL 435007100; 435069100; 435091200; 435252300; 530350000
CC 3-2 (Biochemical Genetics)
Section cross-reference(s): 6, 7, 10

FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	US 2003049686	A1	20030313	US 2002-112455	20020329
	JP 2003102484	A2	20030408	JP 2002-89976	20020327
	DE 10214653	A1	20030327	DE 2002-10214653	20020403
PRAI	KR 2001-55394	A	20010910		

AB The invention provides a method for manufacturing a mutant library of proteins with various sizes and sequences from a parental protein. The invention also provides microorganisms transformed with plasmid vectors containing a recombinant gene prepared by insertion of genomic DNA into a defective template (gene). The invention relates that said insertion of genomic DNA into defective gene is carried out by PCR-coupled recombination or sequence-directed recombination employing a ligase. The invention further provides a process for obtaining said proteins using said transformed microorganisms. Specifically, the invention prepared several defective green fluorescent protein (**GFP**) genes by deletion of nucleotide sequences encoding amino acids of GFPuv, and prepared a defective dihydroorotase gene by deletion of dozens of nucleotides. Recombinant DNAs were constructed by inserting Escherichia coli genomic DNA fragments into the BamHI site of defective genes. A library of microorganisms transformed with plasmids containing said recombinant DNAs was constructed.

ST mutant protein library generation PCR recombination sequence directed; sequence Escherichia peptide insertion defective **GFP** gene; green fluorescent protein gene defective Escherichia genomic DNA recombination; dihydroorotase gene defective Escherichia genomic DNA recombination

IT PCR (polymerase chain reaction)
(-coupled recombination; use of site-directed mutagenesis and PCR-coupled recombination for generation of proteins with various sizes and sequences)

IT Genetic element
RL: BUU (Biological use, unclassified); BIOL (Biological study); USES

(Uses)
(BamHI restriction enzyme recognition site; sequences of various Escherichia coli peptides inserted into BamHI recognition site of defective **GFP** genes, which results in fluorescent proteins of various lengths)

IT Fusion proteins (chimeric proteins)
RL: BPN (Biosynthetic preparation); BSU (Biological study, unclassified); BIOL (Biological study); PREP (Preparation)
(E. coli peptides fused to dihydroorotase; use of site-directed mutagenesis and PCR-coupled recombination for generation of protein with various sizes and sequences having dihydroorotase activity)

IT Escherichia coli
(JM109/pMAL-c2/gfpI5 (KCTC 10058BP), transformed; Escherichia coli transformed with recombinant plasmid vectors containing defective **GFP** genes linked to genomic E. coli DNA)

IT Escherichia coli
(JM109/pMAL-c2/gfpS22 (KCTC 10059BP), transformed; Escherichia coli transformed with recombinant plasmid vectors containing defective **GFP** genes linked to genomic E. coli DNA)

IT Fusion proteins (chimeric proteins)
RL: BPN (Biosynthetic preparation); BSU (Biological study, unclassified); BIOL (Biological study); PREP (Preparation)
(fluorescent, E. coli peptides fused to **GFP**; use of site-directed mutagenesis, PCR-coupled recombination, and/or sequence-directed recombination using ligase for generation of fluorescent proteins with various sizes and sequences)

IT Gene, animal
RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)
(for defective **GFP**, **GFP**Δ **172** -3/176(+2); defective **GFP** genes combined with genomic DNA from Escherichia coli, and use of said recombinant DNA in production of fluorescent proteins of various sizes and sequences)

IT Gene, animal
RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)
(for defective **GFP**, **GFP**Δ176(+2); defective **GFP** genes combined with genomic DNA from Escherichia coli, and use of said recombinant DNA in production of fluorescent proteins of various sizes and sequences)

IT Gene
RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)
(for defective dihydroorotase, DHOΔ68-70(+1); defective dihydroorotase gene, and its linkage to Escherichia coli genomic DNA for use in production of proteins with various sizes and sequences possessing dihydroorotase activity)

IT Proteins
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(green fluorescent; use of site-directed mutagenesis, PCR-coupled recombination, and/or sequence-directed recombination using ligase for generation of fluorescent proteins with various sizes and sequences)

IT Plasmid vectors
(microorganisms transformed with recombinant plasmid vectors containing defective **GFP** genes linked to genomic Escherichia coli DNA, or defective dihydroorotase gene lined to E. coli DNA)

IT Peptide library
(mutant; method for production of mutant library of protein with various sizes and sequences using PCR-coupled recombination or sequence-directed recombination)

IT Nucleic acid library
(of recombinant DNAs; microorganisms transformed with recombinant

plasmid vectors containing defective **GFP** genes linked to genomic Escherichia coli DNA, or defective dihydroorotase gene lined to E. coli DNA)

IT Recombination, genetic
(sequence-directed recombination using ligase; use of site-directed mutagenesis and sequence-directed recombination using ligase for generation of fluorescent proteins with various sizes and sequences)

IT Protein sequences
(sequences of various Escherichia coli peptides inserted into BamHI recognition site of defective **GFP** genes, which results in fluorescent proteins of various lengths)

IT Mutagenesis
(site-directed, deletion; use of site-directed mutagenesis, PCR-coupled recombination, and/or sequence-directed recombination using ligase for generation of proteins with various sizes and sequences)

IT Microorganism
(transformed; microorganisms transformed with recombinant plasmid vectors containing defective **GFP** genes linked to genomic Escherichia coli DNA, or defective dihydroorotase gene lined to E. coli DNA)

IT Molecular cloning
(use of site-directed mutagenesis, PCR-coupled recombination, and/or sequence-directed recombination using ligase for generation of proteins with various sizes and sequences)

IT 501644-98-0 501644-99-1 501645-00-7 501645-01-8 501645-02-9
501645-03-0 501645-04-1 501645-05-2 501645-06-3 501645-07-4
501645-08-5 501730-73-0 501730-74-1 501730-75-2 501730-76-3
501730-77-4 501730-78-5
RL: BUU (Biological use, unclassified); PRP (Properties); BIOL (Biological study); USES (Uses)
(amino acid sequence; sequences of various Escherichia coli peptides inserted into BamHI recognition site of defective **GFP** genes, which results in fluorescent proteins of various lengths)

IT 37353-39-2, Polynucleotide ligase
RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)
(sequence-directed recombination using ligase; use of site-directed mutagenesis and sequence-directed recombination using ligase for generation of fluorescent proteins with various sizes and sequences)

IT 9024-93-5, Dihydroorotase
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(use of site-directed mutagenesis and PCR-coupled recombination for generation of proteins with various sizes and sequences having dihydroorotase activity)

L6 ANSWER 3 OF 11 CAPLUS COPYRIGHT 2004 ACS on STN
AN 2003:296076 CAPLUS
DN 138:315791
ED Entered STN: 17 Apr 2003
TI Fusions of random peptide libraries in scaffold proteins such as green fluorescent protein or β-lactamase
IN Anderson, David; Peelle, Beau Robert; Bogenberger, Jakob Maria
PA Rigel Pharmaceuticals, Inc., USA
SO U.S., 63 pp., Cont.-in-part of U.S. 6,180,343.
CODEN: USXXAM
DT Patent
LA English
IC ICM C07K004-00
ICS C07K014-435; C12Q001-68; C12N015-63; C12N015-12
NCL 530300000; 530350000; 435006000; 435320100; 536023400; 536023500
CC 3-1 (Biochemical Genetics)
Section cross-reference(s): 9

FAN.CNT 4

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	US 6548632	B1	20030415	US 1999-415765	19991008
	US 6180343	B1	20010130	US 1998-169015	19981008
	US 6548249	B1	20030415	US 2000-626581	20000727
	US 6562617	B1	20030513	US 2000-626580	20000727
	US 2001003650	A1	20010614	US 2000-749959	20001227
	US 6596485	B2	20030722		
	US 2003143562	A1	20030731	US 2002-177725	20020620
	US 2003224412	A1	20031204	US 2003-393449	20030318
PRAI	US 1998-169015	A2	19981008		
	US 1999-415765	A3	19991008		
	US 2002-177725	A2	20020620		

AB The invention relates to the use of scaffold proteins, particularly green fluorescent protein (**GFP**) and β-lactamase TEM-1, in fusion constructs with random and defined peptides and peptide libraries. The fusions act to increase the cellular expression levels, decrease the cellular catabolism, increase the conformational stability relative to linear peptides, and increase the steady state concns. of the random peptides and random peptide library members expressed in cells for the purpose of detecting the presence of the peptides and screening random peptide libraries. N-terminal, C-terminal, dual N- and C-terminal, and one or more internal fusions are all contemplated. Internal fusions in Renilla **GFP** may be made in loops 1-5 (amino acid residues 130-135, 154-159, **172**-175, 188-193, or 208-216) for optimal presentation of the peptide. Inclusion of multiple highly flexible amino acid residues between **GFP** and the library allows minimal conformational constraints on the **GFP**. Designed insertion sites in loops 2-4 retain a high level of **GFP** fluorescence when the inserts are flanked by multiple glycines in the tetrapeptide linkers. Novel fusions utilizing self-binding peptides to create a conformationally stabilized fusion domain are also contemplated.

ST scaffold protein fusion random peptide library; green fluorescent protein fusion random peptide library; lactamase fusion random peptide library

IT Animal cell line
(293, **GFP** fusions expressed in; fusions of random peptide libraries in scaffold proteins such as green fluorescent protein or β-lactamase)

IT Aequorea
Renilla reniformis
(**GFP** from; fusions of random peptide libraries in scaffold proteins such as green fluorescent protein or β-lactamase)

IT Animal cell line
(JURKAT, **GFP** fusions expressed in; fusions of random peptide libraries in scaffold proteins such as green fluorescent protein or β-lactamase)

IT Antigen presentation
Peptide library
(fusions of random peptide libraries in scaffold proteins such as green fluorescent protein or β-lactamase)

IT Fusion proteins (chimeric proteins)
RL: ARU (Analytical role, unclassified); BUU (Biological use, unclassified); ANST (Analytical study); BIOL (Biological study); USES (Uses)
(fusions of random peptide libraries in scaffold proteins such as green fluorescent protein or β-lactamase)

IT Linking agents
(glycine-based; fusions of random peptide libraries in scaffold proteins such as green fluorescent protein or β-lactamase)

IT Proteins
RL: ARU (Analytical role, unclassified); BUU (Biological use,

unclassified); ANST (Analytical study); BIOL (Biological study); USES (Uses)
(green fluorescent, enhanced; fusions of random peptide libraries in scaffold proteins such as green fluorescent protein or β-lactamase)

IT Proteins
RL: ARU (Analytical role, unclassified); BUU (Biological use, unclassified); ANST (Analytical study); BIOL (Biological study); USES (Uses)
(green fluorescent; fusions of random peptide libraries in scaffold proteins such as green fluorescent protein or β-lactamase)

IT Fluorescence
(of **GFP** fusions in loops 2-4 with glycine-based linkers; fusions of random peptide libraries in scaffold proteins such as green fluorescent protein or β-lactamase)

IT Proteins
RL: ARU (Analytical role, unclassified); BUU (Biological use, unclassified); ANST (Analytical study); BIOL (Biological study); USES (Uses)
(scaffolding; fusions of random peptide libraries in scaffold proteins such as green fluorescent protein or β-lactamase)

IT Escherichia coli
(β-lactamase TEM-1 from; fusions of random peptide libraries in scaffold proteins such as green fluorescent protein or β-lactamase)

IT 511353-43-8
RL: PRP (Properties)
(Unclaimed; fusions of random peptide libraries in scaffold proteins such as green fluorescent protein or β-lactamase)

IT 9073-60-3, β-Lactamase
RL: ARU (Analytical role, unclassified); BUU (Biological use, unclassified); ANST (Analytical study); BIOL (Biological study); USES (Uses)
(fusions of random peptide libraries in scaffold proteins such as green fluorescent protein or β-lactamase)

IT 76862-68-5, GenBank V00365 136110-02-6, GenBank M58472 138364-37-1, GenBank M60466 138390-43-9, GenBank M65067 139782-89-1, GenBank M15035 139784-89-7, GenBank X02872 139784-99-9, GenBank M15077 139786-07-5, GenBank M25346 139789-49-4, GenBank M26194 139805-89-3, GenBank J00140 139855-08-6, GenBank J00060 139863-26-6, GenBank M86443 140002-89-7, GenBank K01193 140003-58-3, GenBank M15826 140006-57-1, GenBank M32108 140006-61-7, GenBank M10961 140006-78-6, GenBank M55977 140026-45-5, GenBank M16446 140039-08-3, GenBank M11310 140055-20-5, GenBank M29942 140055-22-7, GenBank M29941 140055-23-8, GenBank M29943 140060-13-5, GenBank K02611 140247-47-8, GenBank J01609 140252-19-3, GenBank M22126 140259-58-1, GenBank M25666 140273-28-5, GenBank J04711 140294-17-3, GenBank J03398 140317-11-9, GenBank M60508 140558-28-7, GenBank M13128 140718-03-2, GenBank J01636 140721-25-1, GenBank J01804 140740-40-5, GenBank M38561 140747-82-6, GenBank M34158 140770-54-3, GenBank J04792 140787-38-8, GenBank M62917 140794-75-8, GenBank V00372 141145-61-1, GenBank M26434 141683-91-2, GenBank X58640 144623-65-4, GenBank L04970 145258-01-1, GenBank L06280 147321-20-8, GenBank S48276 148939-74-6, GenBank S61961 151347-01-2, GenBank X74948 151548-08-2, GenBank L25411 151579-06-5, GenBank U03059 152077-65-1, GenBank U03687 152281-89-5, GenBank L26954 152851-60-0, GenBank U04894 153317-13-6, GenBank L26316 158536-81-3, GenBank L36849 163474-68-8, GenBank Z48231 163539-78-4, GenBank L39929 164478-57-3, GenBank L39928 164950-38-3, GenBank U16781 167206-75-9, GenBank U31240 167714-65-0, GenBank U28961 170048-89-2, GenBank U36394 173710-42-4, GenBank L76273 181613-99-0, GenBank U61765 182910-13-0, GenBank U62625 183049-69-6, GenBank U75991 183049-70-9, GenBank U75992 183100-65-4, GenBank Z75185 194082-52-5, GenBank U82227 195650-72-7, GenBank AF012551 196718-14-6, GenBank AF085332

200762-49-8, GenBank AF016891 201951-34-0, GenBank AF025747
203605-36-1, GenBank U28239 205361-40-6, GenBank AJ227752 207106-77-2,
GenBank AB013080 207888-26-4, GenBank AF061748 209138-47-6, GenBank
AF060886 210450-36-5, GenBank AF072538 211159-07-8, GenBank AF073995
224328-20-5, GenBank U62928 225581-17-9, GenBank AB025433 235408-30-7,
GenBank AF170105 235740-40-6, GenBank AF173835 383838-60-6, GenBank
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384662-78-6, GenBank U51019 384971-65-7, GenBank E05448 385206-41-7,
GenBank U62931 389335-68-6, GenBank L37442 389733-83-9, GenBank U89490
392014-96-9, GenBank AF016535 392022-53-6, GenBank U62930 398096-93-0,
GenBank L07486
RL: BSU (Biological study, unclassified); PRP (Properties); BIOL
(Biological study)
(fusions of random peptide libraries in scaffold proteins such as green fluorescent protein or β-lactamase)

IT 511353-44-9 511353-46-1
RL: PRP (Properties)
(unclaimed nucleotide sequence; fusions of random peptide libraries in scaffold proteins such as green fluorescent protein or β-lactamase)

IT 511353-32-5 511353-33-6 511353-34-7 511353-35-8 511353-36-9
511353-37-0 511353-38-1 511353-39-2 511353-40-5 511353-41-6
511353-42-7 511353-45-0 511353-47-2
RL: PRP (Properties)
(unclaimed protein sequence; fusions of random peptide libraries in scaffold proteins such as green fluorescent protein or β-lactamase)

IT 76869-17-5 95088-49-6 105468-12-0 110579-95-8 113516-56-6
115084-19-0 119766-62-0 122580-21-6 135941-52-5 206750-67-6
245759-04-0 245759-06-2 245759-08-4 246862-96-4 246862-97-5
246862-98-6 246862-99-7 246863-01-4 246863-03-6 246863-04-7
246863-05-8 246863-06-9 246863-07-0 246863-08-1 257933-92-9
260055-30-9 265979-95-1 265979-96-2 265979-97-3 265979-98-4
310404-89-8 475270-13-4 475270-18-9 511245-87-7 511245-90-2
511245-92-4 511245-94-6 511245-96-8 511245-98-0 511246-00-7
511246-14-3 511246-19-8 511246-35-8 511246-48-3 511246-51-8
511246-56-3 511246-61-0 511246-66-5
RL: PRP (Properties)
(unclaimed sequence; fusions of random peptide libraries in scaffold proteins such as green fluorescent protein or β-lactamase)

RE.CNT 44 THERE ARE 44 CITED REFERENCES AVAILABLE FOR THIS RECORD
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L6 ANSWER 4 OF 11 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
AN 2004:167884 BIOSIS
DN PREV200400161946
TI Angiogenesis in an in vivo model of adipose tissue development.
AU Neels, Jaap G. [Reprint Author]; Loskutoff, David J. [Reprint Author]
CS Cell Biology, Division of Vascular Biology, Scripps Research Institute, La Jolla, CA, USA
SO Blood, (November 16 2003) Vol. 102, No. 11, pp. 148b. print.
Meeting Info.: 45th Annual Meeting of the American Society of Hematology. San Diego, CA, USA. December 06-09, 2003. American Society of Hematology.
CODEN: BLOOAW. ISSN: 0006-4971.
DT Conference; (Meeting)
Conference; Abstract; (Meeting Abstract)
LA English
ED Entered STN: 24 Mar 2004
Last Updated on STN: 24 Mar 2004
AB Obesity is approaching epidemic levels in Westernized societies, and is associated with an increased risk for cardiovascular disease and cancer. Angiogenesis is a critical component of these pathological processes, and the expanding adipose tissue represents one of the few sites of active angiogenesis in the adult. In spite of the fundamental and clinical importance of angiogenesis in obesity and its associated pathologies, little is known about underlying mechanisms. Although angiogenesis is, historically, the most widely studied mechanism of blood vessel formation, it is now clear that other processes (e.g. endothelial progenitor cell (EPC) recruitment) may also contribute. Angiogenesis is characterized by endothelial sprouting from preexisting blood vessels and it relies on the proliferation, migration, and remodeling of fully differentiated endothelial cells (ECs). In contrast, EPCs are present in the peripheral blood of adult mammals, including humans, and these bone marrow derived EPCs are recruited to target organs and also appear to contribute to the formation of new vessels (vasculogenesis). The absence of suitable animal models has hindered attempts to delineate the importance of these, and possibly other mechanisms of neovascularization during adipose tissue development. In this report, we demonstrate that the 3T3-F442A model of

adipose tissue development (Green, H. and Kehinde, O., J. Cell Physiol. (1979) 101:169-**172**) may be a particularly useful in vivo model to study angiogenesis during adipogenesis. In this model, 3T3-F442A preadipocytes are implanted subcutaneously into athymic Balb/c nude mice. We show that these cells develop into highly vascularized fat pads over the next 14-21 days, and that these fat pads are morphologically indistinguishable from normal adipose tissue. Histological studies demonstrate that a new microvasculature is evident as early as 5 days after cell implantation, and real-time quantitative RT-PCR analyses show that the expression of endothelial markers (e.g. PECAM-1, FLT-4, TIE-1, and TIE-2) and adipogenesis markers (lipoprotein lipase and adipsin) increase in parallel during fat pad development. These results suggest that invasion of endothelial cells, and neovascularization of the fat pads occurs in parallel to adipogenesis during fat pad development. To investigate the potential contribution of EPCs to neovascularization in this model, we grew fat pads in athymic nude mice that had previously received a bone marrow transplantation from transgenic TIE2-**GFP** donor mice. Resulting fat pads were harvested at various times after injection and were stained for PECAM-1 to visualize the vasculature, and for **GFP** to determine the degree of EPC incorporation into it. A PECAM-1 positive neovasculature was observed in the developing fat pads from day 5 on. In spite of this, no **GFP** positive cells were observed in the various sections, the rare exception being those from 14-day old fat pads. The 14-day old fat pads showed a few **GFP** positive endothelial cells lining part of some vessel walls even though analysis by PECAM-1 staining established the presence of an abundant neovasculature. These preliminary studies suggest that the neovasculature originates by sprouting from preexisting, nerve-associated blood vessels located adjacent to the fat pad, and that endothelial progenitor cells only play a minor role in this process.

CC General biology - Symposia, transactions and proceedings 00520
Cytology - Animal 02506
Enzymes - General and comparative studies: coenzymes 10802
Metabolism - General metabolism and metabolic pathways 13002
Nutrition - General studies, nutritional status and methods 13202
Nutrition - Malnutrition and obesity 13203
Cardiovascular system - Physiology and biochemistry 14504

IT Major Concepts
Cardiovascular System (Transport and Circulation); Metabolism; Nutrition

IT Parts, Structures, & Systems of Organisms
adipose tissue; endothelial progenitor cells: circulatory system; microvasculature: circulatory system; neovasculature: circulatory system

IT Diseases
obesity: nutritional disease
Obesity (MeSH)

IT Chemicals & Biochemicals
FLT-4: biomarker; PECAM-1: biomarker; TIE-1: biomarker; TIE-2: biomarker; adipsin: biomarker; lipoprotein lipase [EC 3.1.1.34]: biomarker

IT Methods & Equipment
real-time RT-PCR [real-time reverse transcriptase-polymerase chain reaction]: genetic techniques, laboratory techniques

IT Miscellaneous Descriptors
adipogenesis; angiogenesis; fat pad development

ORGN Classifier
Muridae 86375
Super Taxa
Rodentia; Mammalia; Vertebrata; Chordata; Animalia
Organism Name
3T3-F442A cell line (cell line)

Balb/C mouse (common): transgenic
Taxa Notes
Animals, Chordates, Mammals, Nonhuman Vertebrates, Nonhuman Mammals, Rodents, Vertebrates
RN 37213-56-2Q (adipsin)
60296-01-7Q (adipsin)
9004-02-8 (lipoprotein lipase)
9004-02-8 (EC 3.1.1.34)

L6 ANSWER 5 OF 11 CAPLUS COPYRIGHT 2004 ACS on STN
AN 2002:895268 CAPLUS
DN 138:199159
ED Entered STN: 26 Nov 2002
TI Kallikreins when activating bradykinin B2 receptor induce its redistribution on plasma membrane
AU Hecquet, Claudie; Becker, Robert P.; Tan, Fulong; Erdos, Ervin G.
CS Department of Pharmacology, University of Illinois College of Medicine at Chicago, Chicago, IL, 60612, USA
SO International Immunopharmacology (2002), 2(13-14), 1795-1806
CODEN: IINMBA; ISSN: 1567-5769
PB Elsevier Science B.V.
DT Journal
LA English
CC 2-10 (Mammalian Hormones)
AB The bradykinin (BK) B2 receptor (R) is directly activated by kallikreins and other serine proteases independent of BK release. Both the Gαi and Gαq proteins are involved, shown by the release of arachidonic acid and [Ca2+]i elevation. Site-directed mutagenesis of the receptor and the lack of heterogenous desensitization of the human B2R by the BK and kallikrein emphasize among others the differences between activation by the proteases and the peptide. To characterize further the mechanism whereby kallikreins activate and desensitize the B2R, the authors investigated the distribution of the human B2R tagged with the green fluorescent protein (B2-GFPCt) on the plasma membrane of stably transfected Chinese hamster ovary (CHO) cells. The authors visualized the movement of B2-GFPCt R with confocal fluorescence microscopy after activation by BK or a by serine protease. Continued exposure of the cells to BK led to B2R internalization within 15-20 min. Porcine pancreatic and human recombinant tissue kallikreins induced a rapid definite redistribution of receptors on the plasma membrane within 5 min, prior to internalization. These effects of kallikrein were blocked by the B2R antagonist HOE 140 and by the kallikrein inhibitor, aprotinin. The B2R was also activated by endoproteinases LysC and ArgC and trypsin, but these enzymes did not induce redistribution, only internalization. In control expts., kallikrein had no effect on cells transfected to stably express the angiotensin-converting enzyme-green fluorescent protein (**GFP**). Thus, kallikreins when activating the BK B2R also trigger its redistribution on plasma membrane.
ST kallikrein bradykinin B2 receptor redistribution plasma membrane activation
IT Bradykinin receptors
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(B2; kallikreins and other serine proteases redistribution of bradykinin B2 receptor plasma membrane in receptor activation and involved mechanisms)
IT Biological transport
(calcium; kallikreins and other serine proteases redistribution of bradykinin B2 receptor plasma membrane in receptor activation and involved mechanisms)
IT Artery
(endothelium; kallikreins and other serine proteases redistribution of bradykinin B2 receptor plasma membrane in receptor activation and

involved mechanisms)
IT Biological transport
(internalization; kallikreins and other serine proteases redistribution of bradykinin B2 receptor plasma membrane in receptor activation and involved mechanisms)
IT Cell membrane
Human
(kallikreins and other serine proteases redistribution of bradykinin B2 receptor plasma membrane in receptor activation and involved mechanisms)
IT Biological transport
(redistribution; kallikreins and other serine proteases redistribution of bradykinin B2 receptor plasma membrane in receptor activation and involved mechanisms)
IT 74-79-3, Arginine, biological studies
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(bradykinin B2 receptor residue 169, kallikrein cleavage site; kallikreins and other serine proteases redistribution of bradykinin B2 receptor plasma membrane in receptor activation and involved mechanisms)
IT 56-87-1, Lysine, biological studies
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(bradykinin B2 receptor residue **172**, kallikrein cleavage site; kallikreins and other serine proteases redistribution of bradykinin B2 receptor plasma membrane in receptor activation and involved mechanisms)
IT 58-82-2, Bradykinin 506-32-1, Arachidonic acid 9001-01-8, Kallikrein 9002-07-7, Trypsin 37259-58-8, Serine protease 56645-49-9, Cathepsin G 63551-76-8, Phosphatidylinositol phospholipase C 123175-81-5, Endoproteinase ArgC 123175-82-6, Endoproteinase LysC 389069-73-2, Kallikrein 410538-33-9, Plasma Kallikrein
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(kallikreins and other serine proteases redistribution of bradykinin B2 receptor plasma membrane in receptor activation and involved mechanisms)
IT 7440-70-2, Calcium, biological studies
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(transport; kallikreins and other serine proteases redistribution of bradykinin B2 receptor plasma membrane in receptor activation and involved mechanisms)
RE.CNT 24 THERE ARE 24 CITED REFERENCES AVAILABLE FOR THIS RECORD
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L6 ANSWER 6 OF 11 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
DUPLICATE 1
AN 2002:219934 BIOSIS
DN PREV200200219934
TI Transient expression of fluorescent tau proteins promotes process formation in PC12 cells: Contributions of the tau C-terminus to this process.
AU Yu, Jiang-Zhou; Kuret, Jeff; Rasenick, Mark M. [Reprint author]
CS Department of Physiology and Biophysics, University of Illinois at Chicago College of Medicine, 835 S. Wolcott Ave., Room E202, Chicago, IL, 60612-7342, USA
raz@uic.edu
SO Journal of Neuroscience Research, (March 1, 2002) Vol. 67, No. 5, pp. 625-633. print.
CODEN: JNREDK. ISSN: 0360-4012.
DT Article
LA English
ED Entered STN: 27 Mar 2002
Last Updated on STN: 27 Mar 2002
AB The neuronal microtubule-associated protein tau promotes microtubule assembly and has been implicated in the development of axonal morphology. In this study, PC12 cells were transiently transfected with constructs coding fusion proteins of human tau with green fluorescent protein (**GFP**). Expression of tau constructs actively stabilized microtubules. Expression of the C-terminus of tau can mimic this effect in living cells, though to a lesser extent because of the absence of the tau N-terminus. However, tau colocalization with microtubules did not require the presence of the tau N-terminus. Transient expression of tau (including tau24, a four-repeat human tau isoform encoded in 383 residues, and tau23, human fetal tau isoform encoded in 352 residues) stimulated process formation in PC12 cells, and this occurred faster with tau24 than with tau23. The residues (residues 154-**172** in tau23) that confer microtubule nucleation activity of tau in vitro are not required for tau-directed process formation. However, when tau induces the formation of cellular processes in response to cortical breakdown by cytochalasin B, residues 154-**172** must be present. Thus, it appears that tau may serve to promote cellular process outgrowth in cultured neuronal cells and that C-terminus of tau is essential to this process.
CC Cytology - Animal 02506
Muscle - Physiology and biochemistry 17504
Nervous system - Physiology and biochemistry 20504
IT Major Concepts
Nervous System (Neural Coordination)
IT Parts, Structures, & Systems of Organisms
microtubule: muscular system; neuronal cell: nervous system
IT Chemicals & Biochemicals
cytochalasin B; tau protein; tau23; tau24
ORGN Classifier
Muridae 86375
Super Taxa
Rodentia; Mammalia; Vertebrata; Chordata; Animalia
Organism Name
PC12 cell line
Taxa Notes
Animals, Chordates, Mammals, Nonhuman Vertebrates, Nonhuman Mammals, Rodents, Vertebrates

RN 14930-96-2 (cytochalasin B)

L6 ANSWER 7 OF 11 CAPLUS COPYRIGHT 2004 ACS on STN
AN 2002:858987 CAPLUS
DN 138:149221
ED Entered STN: 13 Nov 2002
TI Development of a positive genetic selection system for inhibition of protein splicing using mycobacterial inteins in Escherichia coli DNA gyrase subunit A
AU Adam, Eric; Perler, Francine B.
CS New England BioLabs, Beverly, MA, 01915, USA
SO Journal of Molecular Microbiology and Biotechnology (2002), 4(5), 479-487
CODEN: JMMBFF; ISSN: 1464-1801
PB Horizon Scientific Press
DT Journal
LA English
CC 6-3 (General Biochemistry)
Section cross-reference(s): 3, 10
AB An intein-based pos. genetic selection system was developed to study protein splicing and to provide a selection system with the potential for finding splicing inhibitors. Inteins can be novel anti-microbial targets when present in essential proteins since blocking splicing would kill the organism. For example, pathogenic mycobacteria encode inteins that interrupt DNA gyrase. The gyrase selection system exploits (1) splicing of inteins out of Gyrase A and (2) the dominant lethal effect of quinolone poisoning of DNA gyrase, which in turn blocks replication. The system was adapted for whole-cell high-throughput screening using green fluorescent protein as an automatable readout of viability. To demonstrate the efficacy of this system, mutations that blocked splicing of the Mycobacterium xenopi Gyrase A intein were isolated. Splicing was then assayed at a second temperature to identify inteins with a temperature-sensitive splicing phenotype. Mutations were mapped onto a structure-based sequence alignment, which led to the rational prediction of a temperature-sensitive splicing mutation. GyrA intein subdomain relationships also provided insight into intein evolution.
ST Mycobacterium DNA gyrase A intein protein splicing mutant screening; protein intein splicing inhibitor genetic selection Escherichia gene gyrA
IT Enzymes, biological studies
RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)
(DNA gyrases; development of a pos. genetic selection system for inhibition of protein splicing using mycobacterial inteins in Escherichia coli DNA gyrase subunit A)
IT Escherichia coli
High throughput screening
Mycobacterium flavescens
Mycobacterium gordonae
Mycobacterium kansasii
Mycobacterium leprae
Mycobacterium malmoense
Mycobacterium xenopi
Protein splicing
(development of a pos. genetic selection system for inhibition of protein splicing using mycobacterial inteins in Escherichia coli DNA gyrase subunit A)
IT Inteins
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(development of a pos. genetic selection system for inhibition of protein splicing using mycobacterial inteins in Escherichia coli DNA gyrase subunit A)
IT Growth, microbial

(development of a pos. genetic selection system for inhibition of protein splicing using mycobacterial inteins in Escherichia coli DNA gyrase subunit A and dominant lethal effect of quinonolones on gyrase)

IT Reporter gene
RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)
(for **GFP** (green fluorescent protein); development of a pos. genetic selection system for inhibition of protein splicing using mycobacterial inteins in Escherichia coli DNA gyrase subunit A)

IT Proteins
RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)
(green fluorescent, reporter; development of a pos. genetic selection system for inhibition of protein splicing using mycobacterial inteins in Escherichia coli DNA gyrase subunit A)

IT Gene, microbial
RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)
(gyrA96, ofloxacin-resistant; development of a pos. genetic selection system for inhibition of protein splicing using mycobacterial inteins in Escherichia coli DNA gyrase subunit A and dominant lethal effect of quinonolones on gyrase)

IT Gene, microbial
RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)
(gyrA; development of a pos. genetic selection system for inhibition of protein splicing using mycobacterial inteins in Escherichia coli DNA gyrase subunit A)

IT Structure-activity relationship
(intein protein splicing-inhibiting; development of a pos. genetic selection system for inhibition of protein splicing using mycobacterial inteins in Escherichia coli DNA gyrase subunit A and isolation of intein mutations)

IT 82419-36-1, Ofloxacin
RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)
(gyrase inhibitor; development of a pos. genetic selection system for inhibition of protein splicing using mycobacterial inteins in Escherichia coli DNA gyrase subunit A and dominant lethal effect of quinonolones on gyrase)

IT 56-41-7, L-Alanine, biological studies
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(residue **172** of intein; development of a pos. genetic selection system for inhibition of protein splicing using mycobacterial inteins in Escherichia coli DNA gyrase subunit A and isolation of intein mutations)

IT 56-45-1, L-Serine, biological studies
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(residue 53 of intein; development of a pos. genetic selection system for inhibition of protein splicing using mycobacterial inteins in Escherichia coli DNA gyrase subunit A and isolation of intein mutations)

IT 56-86-0, L-Glutamic acid, biological studies
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(residue 65 of intein; development of a pos. genetic selection system for inhibition of protein splicing using mycobacterial inteins in Escherichia coli DNA gyrase subunit A and isolation of intein mutations)

IT 73-32-5, L-Isoleucine, biological studies
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(residues 21 and 97 of intein; development of a pos. genetic selection system for inhibition of protein splicing using mycobacterial inteins

in Escherichia coli DNA gyrase subunit A and isolation of intein mutations)

IT 72-19-5, L-Threonine, biological studies
RL: BSU (Biological study, unclassified); BIOL (Biological study) (residues 3, 72, and 184 of intein; development of a pos. genetic selection system for inhibition of protein splicing using mycobacterial inteins in Escherichia coli DNA gyrase subunit A and isolation of intein mutations)

RE.CNT 30 THERE ARE 30 CITED REFERENCES AVAILABLE FOR THIS RECORD

RE

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L6 ANSWER 8 OF 11 CAPLUS COPYRIGHT 2004 ACS on STN
AN 2001:779210 CAPLUS
DN 136:304984
ED Entered STN: 26 Oct 2001
TI DNA substrates influence the recombination efficiency mediated by FLP recombinase expressed in mammalian cells

AU Nakano, Masakazu; Ishimura, Masakazu; Chiba, Joe; Kanegae, Yumi; Saito, Izumu
CS Laboratory of Molecular Genetics, Institute of Medical Science, The University of Tokyo, Tokyo, 108-8639, Japan

SO Microbiology and Immunology (2001), 45(9), 657-665
CODEN: MIIMDV; ISSN: 0385-5600
PB Center for Academic Publications Japan
DT Journal
LA English
CC 3-4 (Biochemical Genetics)
Section cross-reference(s): 7

AB The FLP recombinase derived from Saccharomyces cerevisiae mediates precise site-specific recombination between a pair of FLP recognition targets (FRTs). Like the Cre/loxP system derived from bacteriophage P1, the FLP/FRT system has recently been applied to gene regulation systems using

an FLP-expressing recombinant adenovirus (rAd). In an attempt to improve the FLP/FRT system by altering its DNA substrates, we compared the recombination efficiency among different substrates by a quant. in vitro assay using FLP expressed in mammalian cells. Unexpectedly, we found that one linearized DNA substrate showed 4- to > 20-fold lower recombination efficiency than other substrates, which phenomenon has not been observed in the Cre/loxP system. The quant. in vitro assay using truncated DNA substrates suggested that the recombination efficiency seemed to be influenced not only by the linearized position of the substrate, but also by the length between a pair of FRTs. Such substrate preference of FLP expressed in mammalian cells should probably be noted when designing versatile applications of the FLP/FRT system as a gene regulation system in mammalian systems. Fortunately, however, we demonstrated that no substrate preference was observed when using a particular substrate (pCAFNF5) and the preference was reduced when using a certain pair of mutant FRTs (**172**), which will also be a promising tool for simultaneous gene regulation in combination with wild-type FRT.

ST Saccharomyces FLP recognition target sequence recombinase; DNA substrate genetic recombination FLP recombinase

IT Saccharomyces cerevisiae
(DNA substrates influence the recombination efficiency mediated by FLP recombinase expressed in mammalian cells)

IT Enzymes, biological studies
RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)
(DNA-recombining, gene FLP; DNA substrates influence the recombination efficiency mediated by FLP recombinase expressed in mammalian cells)

IT Genetic element
RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)
(FRT (FLP recognition target); DNA substrates influence the recombination efficiency mediated by FLP recombinase expressed in mammalian cells)

IT DNA sequences
(FRT; DNA substrates influence the recombination efficiency mediated by FLP recombinase expressed in mammalian cells)

IT Reporter gene
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(**GFP** and LacZ; DNA substrates influence the recombination efficiency mediated by FLP recombinase expressed in mammalian cells)

IT Structure-activity relationship
(enzyme substrate; DNA substrates influence the recombination efficiency mediated by FLP recombinase expressed in mammalian cells)

IT Animal cell
(mammalian; DNA substrates influence the recombination efficiency mediated by FLP recombinase expressed in mammalian cells)

IT Transgene
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(pCAFNF5 with **GFP** and LacZ; DNA substrates influence the recombination efficiency mediated by FLP recombinase expressed in mammalian cells)

IT Plasmids
(pCAFNF5; DNA substrates influence the recombination efficiency mediated by FLP recombinase expressed in mammalian cells)

IT Recombination, genetic
(site-specific; DNA substrates influence the recombination efficiency mediated by FLP recombinase expressed in mammalian cells)

IT 410802-79-8
RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)
(nucleotide sequence; DNA substrates influence the recombination efficiency mediated by FLP recombinase expressed in mammalian cells)

RE.CNT 23 THERE ARE 23 CITED REFERENCES AVAILABLE FOR THIS RECORD
RE
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L6 ANSWER 9 OF 11 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
DUPLICATE 2
AN 2001:538663 BIOSIS
DN PREV200100538663
TI Green fluorescent protein rendered susceptible to proteolysis: Positions for protease-sensitive insertions.
AU Chiang, Cheng-Feng; Okou, David T.; Griffin, Tony B.; Verret, C. Reynold; Williams, Myron N. V. [Reprint author]
CS Department of Chemistry, Clark Atlanta University, Atlanta, GA, 30314, USA
mnwill@cau.edu
SO Archives of Biochemistry and Biophysics, (October 15, 2001) Vol. 394, No. 2, pp. 229-235. print.
CODEN: ABBIA4. ISSN: 0003-9861.
DT Article
LA English
ED Entered STN: 21 Nov 2001
Last Updated on STN: 25 Feb 2002
AB The green fluorescent protein (**GFP**) is highly resistant to proteolysis and remains uncleaved after prolonged incubation with trypsin or pronase despite several putative tryptic and chymotryptic sites in exposed loops. We have rendered **GFP** sensitive to proteolysis by inserting five amino acids, IEGRS, in loops at position 157, **172**, or 189. Excitation and emission maxima of the three insertion mutants were similar to those of wild type, but quantum yields of mutants OMEGA172 and OMEGA189 were lower, indicating increased freedom of the fluorophore. Trypsin cleaved the native (folded) form of each mutant at a unique site defined by the insert. Pronase also yields similar digestion patterns in these variants, but further proteolysis was also observed, suggesting that the primary cleavage relaxes **GFP** structure and reveals previously inaccessible sites. Fluorescence of OMEGA189 changed little upon digestion with trypsin but decreased progressively by as much as 40% upon digestion with increasing amounts of pronase. Fluorescence of other variants was not affected significantly by the proteases, further confirming the remarkable stabilities of **GFP** variants. These constructs define a new conformation-sensitive site around residue 189 of **GFP** and show that **GFP** may be useful for design of

protease-susceptible molecules for monitoring of specific proteolytic activities in vivo.

CC Biochemistry studies - General 10060
Biochemistry studies - Proteins, peptides and amino acids 10064
Enzymes - General and comparative studies: coenzymes 10802
Physiology and biochemistry of bacteria 31000

IT Major Concepts
Biochemistry and Molecular Biophysics

IT Chemicals & Biochemicals
factor Xa; green fluorescent protein; pronase; protease; trypsin

IT Methods & Equipment
mutagenesis: analytical method, genetic engineering, genetic method

IT Miscellaneous Descriptors
protease-sensitive insertions; proteolysis

ORGN Classifier
Bacteria 05000
Super Taxa
Microorganisms
Organism Name
bacteria
Taxa Notes
Bacteria, Eubacteria, Microorganisms

RN 9002-05-5 (factor Xa)
9036-06-0 (pronase)
9001-92-7 (protease)
9002-07-7 (trypsin)

L6 ANSWER 10 OF 11 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
DUPLICATE 3

AN 2000:129563 BIOSIS

DN PREV200000129563

TI Post-translational regulation of Adr1 activity is mediated by its DNA binding domain.

AU Sloan, James S.; Dombeck, Kenneth M.; Young, Elton T. [Reprint author]

CS Department of Biochemistry, University of Washington, Seattle, WA, 98195-7350, USA

SO Journal of Biological Chemistry, (Dec. 31, 1999) Vol. 274, No. 53, pp. 37575-37582. print.
CODEN: JBCHA3. ISSN: 0021-9258.

DT Article

LA English

ED Entered STN: 12 Apr 2000
Last Updated on STN: 4 Jan 2002

AB ADR1 encodes a transcriptional activator that regulates genes involved in carbon source utilization in Saccharomyces cerevisiae. ADR1 is itself repressed by glucose, but the significance of this repression for regulating target genes is not known. To test if the reduction in Adr1 levels contributes to glucose repression of ADH2 expression, we generated yeast strains in which the level of Adr1 produced during growth in glucose-containing medium is similar to that present in wild-type cells grown in the absence of glucose. In these Adr1-overproducing strains, ADH2 expression remained tightly repressed, and UAS1, the element in the ADH2 promoter that binds Adr1, was sufficient to maintain glucose repression. Post-translational modification of Adr1 activity is implicated in repression, since ADH2 derepression occurred in the absence of de novo protein synthesis. The N-terminal **172** amino acids of Adr1, containing the DNA binding and nuclear localization domains, fused to the Herpesvirus VP16-encoded transcription activation domain, conferred regulated expression at UAS1. Nuclear localization of an Adr1-**GFP** fusion protein was not glucose-regulated, suggesting that the DNA binding domain of Adr1 is sufficient to confer regulated expression on target genes. A Gal4-Adr1 fusion protein was unable to confer glucose repression

at GAL4-dependent promoters, suggesting that regulation mediated by ADR1 is specific to UAS1.

CC Genetics - Plant 03504
Biochemistry studies - General 10060

IT Major Concepts
Molecular Genetics (Biochemistry and Molecular Biophysics)

IT Chemicals & Biochemicals
Adr1 protein: DNA binding domain, activity, post-translational regulation; Saccharomyces cerevisiae ADH2 gene

L6 ANSWER 11 OF 11 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
DUPLICATE 4

AN 1998:509211 BIOSIS

DN PREV199800509211

TI UGA codon position affects the efficiency of selenocysteine incorporation into glutathione peroxidase-1.

AU Wen, Wu; Weiss, Sherri L.; Sunde, Roger A. [Reprint author]

CS 217 Gwynn Hall, Univ. Missouri, Columbia, MO 65211, USA

SO Journal of Biological Chemistry, (Oct. 23, 1998) Vol. 273, No. 43, pp. 28533-28541. print.
CODEN: JBCHA3. ISSN: 0021-9258.

DT Article

LA English

ED Entered STN: 18 Dec 1998
Last Updated on STN: 10 May 1999

AB AUGA codon and a selenocysteine insertion sequence in the 3'-untranslated region are the only established mRNA elements necessary for selenocysteine (Sec or U) incorporation during translation. These two elements, however, do not universally confer efficient Sec incorporation. The objective of this study was to systematically examine the effect of UGA codon position on efficiency of Sec insertion. In a glutathione peroxidase-1 (F-GPX1) expression vector, the UGA at the native position (U47) was mutated to a cysteine codon, and codons for Ser-7, Ser-12, Ser-18, Ser-29, Ser-45, Ser-93, Cys-154, Val-**172**, Ser-178, and Ser-195 were individually mutated to UGA and transiently expressed in COS-7 cells. 75Se incorporation at the 11 positions was 31, 72, 54, 105, 90, 100, 146, 135, 13, 11, and 43%, respectively, of 75Se incorporation at U47, suggesting that Sec is more efficiently incorporated at UGA codons positioned in the middle of the coding region rather than close to the 5' or 3' ends. Ribonuclease protection showed that these differences were not due to differences in mRNA level. When the green fluorescence protein (**GFP**) coding region was placed in-frame at the 5' or 3' ends of the coding region in F-GPX1 to produce chimeric 50-51-kDa **GFP**/GPX1 proteins, Sec incorporation at UGA codons, formerly close to the 5' or 3' ends, was increased to levels comparable to the UGA at U47. Insertion of **GFP** after the UAA-stop was just as effective in increasing Sec insertion efficiency as **GFP** inserted before the stop. These studies used a recombinant expression model that incorporated See at non-native UGA codons at rates equal to those of endogenous glutathione peroxidase-1 and showed that the efficiency of Sec incorporation can be modulated by UGA position; Sec incorporation at high efficiency appears to require that the UGA be >21 nucleotides from the AUG-start and >204 nucleotides from the selenocysteine insertion sequence element.

CC Enzymes - General and comparative studies: coenzymes 10802
Cytology - Animal 02506
Biochemistry methods - General 10050
Biochemistry studies - General 10060

IT Major Concepts
Enzymology (Biochemistry and Molecular Biophysics); Methods and Techniques

IT Chemicals & Biochemicals
glutathione peroxidase-1: analysis; selenocysteine: analysis,

ANSWER 1 OF 1 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
DUPLICATE 1
AN 1997:344876 BIOSIS
DN PREV199799644079
TI An improved GFP cloning cassette designed for prokaryotic transcriptional fusions.
AU Miller, William G.; Lindow, Steven E. [Reprint author]
CS Dep. Plant Microbial Biol., Univ. Calif., 111 Koshland Hall, Berkeley, CA 94720, USA
SO Gene (Amsterdam), (1997) Vol. 191, No. 2, pp. 149-153.
CODEN: GENED6. ISSN: 0378-1119.
June 1997
DT Article
LA English
ED Entered STN: 11 Aug 1997
Last Updated on STN: 11 Aug 1997
AB A new gfp cloning cassette designed for prokaryotic transcriptional fusions has been constructed. This cassette consists of gfp (containing the S65T 'red-shift' (Heim et al. (1995) Nature 373, 663-6641 and F64L 'protein solubility' (Cormack et al. (1996) Gene **173**, 33-38) mutations) flanked by convenient restriction sites, a translational enhancer, and a consensus ribosome binding site with an optimized spacer region. gfp fusion strains containing this cassette demonstrate from 40- to 80-fold greater fluorescence intensity than **wild-type** **gfp** fusion strains. Additionally, this cassette confers sufficient fluorescence to recipient cells to be used in low copy-number plasmids, with promoters conferring low levels of transcription, and in bacterial taxa other than Escherichia, such as Pseudomonas.
CC Genetics - General 03502
Biochemistry studies - Nucleic acids, purines and pyrimidines 10062
Replication, transcription, translation 10300
Physiology and biochemistry of bacteria 31000
Genetics of bacteria and viruses 31500
IT Major Concepts
Biochemistry and Molecular Biophysics; Genetics; Molecular Genetics (Biochemistry and Molecular Biophysics); Physiology
IT Miscellaneous Descriptors
CONSENSUS RIBOSOME BINDING SITE; CONSTRUCTION; DESIGN; GENETIC TOOL; GREEN FLUORESCENT PROTEIN CLONING CASSETTE; GREEN FLUORESCENT PROTEIN GENE; MOLECULAR GENETICS; OPTIMIZER SPACER REGION; PGREENTIR; PLASMID; PROMOTER; REPORTER GENE; RESTRICTION SITES; TRANSCRIPTIONAL FUSION; TRANSCRIPTIONAL REGULATION; TRANSLATIONAL ENHANCER
ORGN Classifier
Enterobacteriaceae 06702
Super Taxa
Facultatively Anaerobic Gram-Negative Rods; Eubacteria; Bacteria; Microorganisms
Organism Name
Escherichia coli
Taxa Notes
Bacteria, Eubacteria, Microorganisms
ORGN Classifier
Pseudomonadaceae 06508
Super Taxa
Gram-Negative Aerobic Rods and Cocci; Eubacteria; Bacteria; Microorganisms
Organism Name
Pseudomonas syringae
Taxa Notes
Bacteria, Eubacteria, Microorganisms

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ANSWER 1 OF 1 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
DUPLICATE 1
AN 1997:344876 BIOSIS
DN PREV199799644079
TI An improved GFP cloning cassette designed for prokaryotic transcriptional fusions.
AU Miller, William G.; Lindow, Steven E. [Reprint author]
CS Dep. Plant Microbial Biol., Univ. Calif., 111 Koshland Hall, Berkeley, CA 94720, USA
SO Gene (Amsterdam), (1997) Vol. 191, No. 2, pp. 149-153.
CODEN: GENED6. ISSN: 0378-1119.
DT Article
LA English
ED Entered STN: 11 Aug 1997
Last Updated on STN: 11 Aug 1997
AB A new gfp cloning cassette designed for prokaryotic transcriptional fusions has been constructed. This cassette consists of gfp (containing the S65T 'red-shift' (Heim et al. (1995) Nature 373, 663-6641 and F64L 'protein solubility' (Cormack et al. (1996) Gene **173**, 33-38) mutations) flanked by convenient restriction sites, a translational enhancer, and a consensus ribosome binding site with an optimized spacer region. gfp fusion strains containing this cassette demonstrate from 40- to 80-fold greater fluorescence intensity than **wild-type** **gfp** fusion strains. Additionally, this cassette confers sufficient fluorescence to recipient cells to be used in low copy-number plasmids, with promoters conferring low levels of transcription, and in bacterial taxa other than Escherichia, such as Pseudomonas.
CC Genetics - General 03502
Biochemistry studies - Nucleic acids, purines and pyrimidines 10062
Replication, transcription, translation 10300
Physiology and biochemistry of bacteria 31000
Genetics of bacteria and viruses 31500
IT Major Concepts
Biochemistry and Molecular Biophysics; Genetics; Molecular Genetics (Biochemistry and Molecular Biophysics); Physiology
IT Miscellaneous Descriptors
CONSENSUS RIBOSOME BINDING SITE; CONSTRUCTION; DESIGN; GENETIC TOOL; GREEN FLUORESCENT PROTEIN CLONING CASSETTE; GREEN FLUORESCENT PROTEIN GENE; MOLECULAR GENETICS; OPTIMIZER SPACER REGION; PGREENTIR; PLASMID; PROMOTER; REPORTER GENE; RESTRICTION SITES; TRANSCRIPTIONAL FUSION; TRANSCRIPTIONAL REGULATION; TRANSLATIONAL ENHANCER
ORGN Classifier
Enterobacteriaceae 06702
Super Taxa
Facultatively Anaerobic Gram-Negative Rods; Eubacteria; Bacteria; Microorganisms
Organism Name
Escherichia coli
Taxa Notes
Bacteria, Eubacteria, Microorganisms
ORGN Classifier
Pseudomonadaceae 06508
Super Taxa
Gram-Negative Aerobic Rods and Cocci; Eubacteria; Bacteria; Microorganisms
Organism Name
Pseudomonas syringae
Taxa Notes
Bacteria, Eubacteria, Microorganisms

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     (FILE 'HOME' ENTERED AT 14:57:26 ON 26 APR 2004)

     FILE 'BIOSIS, CAPLUS, EMBASE, MEDLINE, CANCERLIT, JAPIO' ENTERED AT
     14:58:00 ON 26 APR 2004
L1           301 S (WILD TYPE GFP)
L2             0 S L1 AND 157
L3             0 S L1 AND 157
L4             0 S L1 AND 158
L5             0 S L1 AND 172
L6             3 S L1 AND 173
L7             1 DUPLICATE REMOVE L6 (2 DUPLICATES REMOVED)

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(FILE 'HOME' ENTERED AT 14:57:26 ON 26 APR 2004)

FILE 'BIOSIS, CAPLUS, EMBASE, MEDLINE, CANCERLIT, JAPIO' ENTERED AT 14:58:00 ON 26 APR 2004

L1 301 S (WILD TYPE GFP)
L2 0 S L1 AND 157
L3 0 S L1 AND 157
L4 0 S L1 AND 158
L5 0 S L1 AND 172
L6 3 S L1 AND 173
L7 1 DUPLICATE REMOVE L6 (2 DUPLICATES REMOVED)

=>

ANSWER 1 OF 2 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
AN 1997:174907 BIOSIS
DN PREV199799466620
TI A novel mutation which enhances the fluorescence of green fluorescent **protein** at high temperatures.
AU Kimata, Yukio; Iwaki, Masaharu; Lim, Chun Ren; Kohno, Kenji [Reprint author]
CS Res. Education Cent. Genetic Information, Nara Inst. Sci. Technol., 8916-5, Takayama, Ikoma, Nara 630-01, Japan
SO Biochemical and Biophysical Research Communications (1997) Vol. 232, No. 1, pp. 69-73.
CODEN: BBRCA9. ISSN: 0006-291X.
DT Article
LA English
ED Entered STN: 24 Apr 1997
Last Updated on STN: 24 Apr 1997
AB Green fluorescent **protein** (GFP) from Aequorea victoria is widely used as a marker of gene expression and **protein** localization in living cells from **prokaryotes** to eukaryotes. However, the total fluorescent signal from **wild-type GFP** is very weak when expressed in cells cultured at 37 degree C compared to 30 degree C or below. This characteristic makes GFP poorly suited to use as a marker in mammalian cells. Here we describe a new variant of GFP which carries a substitution of **Ser-147** to **Pro** (S147P GFP) and which emits a stronger fluorescent signal than the **wild-type GFP** at high temperature. When S147P is combined with the **Ser**-65 to Thr mutation (S65T GFP), the resulting double mutant emits fluorescence which is several-fold stronger than GFP with a single S65T modification in both bacterial or mammalian cells. This S147P mutation should be useful for constructing new GFP variants which stably emit strong fluorescence at a wide range of culturing temperatures.
CC Genetics - Animal 03506
Biochemistry studies - General 10060
Biophysics - General 10502
Temperature - General measurement and methods 23001
IT Major Concepts
Biochemistry and Molecular Biophysics; Genetics; Methods and Techniques
IT Miscellaneous Descriptors
FLUORESCENCE; GREEN FLUORESCENT **PROTEIN**; HIGH TEMPERATURES; MOLECULAR GENETICS; MUTANT; MUTATION; WILD-TYPE
ORGN Classifier
Cnidaria 41000
Super Taxa
Invertebrata; Animalia
Organism Name
Aequorea victoria
Taxa Notes
Animals, Invertebrates

L4 ANSWER 2 OF 2 JAPIO (C) 2004 JPO on STN
AN 1998-234382 JAPIO
TI FLUORESCENT **PROTEIN**
IN KONO KENJI; TAKEDA KATSUO; HASEGAWA MAMORU
PA DEINABETSUKU KENKYUSHO:KK
PI JP 10234382 A 19980908 Heisei
AI JP 1997-62370 (JP09062370 Heisei) 19970227
PRAI JP 1997-62370 19970227
SO PATENT ABSTRACTS OF JAPAN (CD-ROM), Unexamined Applications, Vol. 1998
IC ICM C12N015-09
ICS C07H021-04; C07K014-435; C12N001-21; C12N005-10; C12P021-02; C12Q001-68

ANSWER 1 OF 2 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
AN 1997:174907 BIOSIS
DN PREV199799466620
TI A novel mutation which enhances the fluorescence of green fluorescent **protein** at high temperatures.
AU Kimata, Yukio; Iwaki, Masaharu; Lim, Chun Ren; Kohno, Kenji [Reprint author]
CS Res. Education Cent. Genetic Information, Nara Inst. Sci. Technol., 8916-5, Takayama, Ikoma, Nara 630-01, Japan
SO Biochemical and Biophysical Research Communications, (1997) Vol. 232, No. 1, pp. 69-73.
CODEN: BBRCA9. ISSN: 0006-291X.
DT Article
LA English
ED Entered STN: 24 Apr 1997
Last Updated on STN: 24 Apr 1997
AB Green fluorescent **protein** (GFP) from Aequorea victoria is widely used as a marker of gene expression and **protein** localization in living cells from **prokaryotes** to eukaryotes. However, the total fluorescent signal from **wild-type GFP** is very weak when expressed in cells cultured at 37 degree C compared to 30 degree C or below. This characteristic makes GFP poorly suited to use as a marker in mammalian cells. Here we describe a new variant of GFP which carries a substitution of **Ser-147** to **Pro** (S147P GFP) and which emits a stronger fluorescent signal than the **wild-type GFP** at high temperature. When S147P is combined with the **Ser**-65 to Thr mutation (S65T GFP), the resulting double mutant emits fluorescence which is several-fold stronger than GFP with a single S65T modification in both bacterial or mammalian cells. This S147P mutation should be useful for constructing new GFP variants which stably emit strong fluorescence at a wide range of culturing temperatures.
CC Genetics - Animal 03506
Biochemistry studies - General 10060
Biophysics - General 10502
Temperature - General measurement and methods 23001
IT Major Concepts
Biochemistry and Molecular Biophysics; Genetics; Methods and Techniques
IT Miscellaneous Descriptors
FLUORESCENCE; GREEN FLUORESCENT **PROTEIN**; HIGH TEMPERATURES; MOLECULAR GENETICS; MUTANT; MUTATION; WILD-TYPE
ORGN Classifier
Cnidaria 41000
Super Taxa
Invertebrata; Animalia
Organism Name
Aequorea victoria
Taxa Notes
Animals, Invertebrates

L4 ANSWER 2 OF 2 JAPIO (C) 2004 JPO on STN
AN 1998-234382 JAPIO
TI FLUORESCENT **PROTEIN**
IN KONO KENJI; TAKEDA KATSUO; HASEGAWA MAMORU
PA DEINABETSUKU KENKYUSHO:KK
PI JP 10234382 A 19980908 Heisei
AI JP 1997-62370 (JP09062370 Heisei) 19970227
PRAI JP 1997-62370 19970227
SO PATENT ABSTRACTS OF JAPAN (CD-ROM), Unexamined Applications, Vol. 1998
IC ICM C12N015-09
ICS C07H021-04; C07K014-435; C12N001-21; C12N005-10; C12P021-02; C12Q001-68

ICI C12N001-21, C12R001:19; C12N005-10, C12R001:91; C12P021-02, C12R001:19

AB **PROBLEM** TO BE SOLVED: To obtain a fluorescent **protein** capable of being expressed even by the culture of a host cell at a high temperature (37°C), emitting stronger fluorescent light than those of conventional fluorescent **proteins** (GFP), and useful as a labeling agent for the analyses of **protein** localization in live cells, a reporter for the analyses of **promoters**, etc., by introducing two mutation amino acids into a **wild type GFP**.

SOLUTION: This fluorescent **protein** is obtained by mutating the Number **147 serine** and the Number 65 **serine** of the cDNA of a **wild type GFP** with **proline** and threonine, respectively, by a site-specific mutation method, etc., transforming Escherichia coil with a plasmid containing the obtained GFPcDNA and subsequently expressing the mutated GFP containing an amino acid sequence of the formula in the Escherichia coil at a high temperature (37°C). The fluorescent **protein** emits about three-fold fluorescent light that of S65T mutant, is contained in a higher concentration than that of the S65T mutant, when expressed in the cell, and emits the fluorescent light under a high temperature (37°C).

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ANSWER 1 OF 1 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
DUPLICATE 1
AN 2001:538663 BIOSIS
DN PREV200100538663
TI Green fluorescent protein rendered susceptible to proteolysis: Positions for protease-sensitive insertions.
AU Chiang, Cheng-Feng; Okou, David T.; Griffin, Tony B.; Verret, C. Reynold; Williams, Myron N. V. [Reprint author]
CS Department of Chemistry, Clark Atlanta University, Atlanta, GA, 30314, USA
mnwill@cau.edu
SO Archives of Biochemistry and Biophysics, (October 15, 2001) Vol. 394, No. 2, pp. 229-235. print.
CODEN: ABBIA4. ISSN: 0003-9861.
DT Article
LA English
ED Entered STN: 21 Nov 2001
Last Updated on STN: 25 Feb 2002
AB The green fluorescent protein (**GFP**) is highly resistant to proteolysis and remains uncleaved after prolonged incubation with trypsin or pronase despite several putative tryptic and chymotryptic sites in exposed loops. We have rendered **GFP** sensitive to proteolysis by inserting five amino acids, IEGRS, in loops at position **157**, **172**, or 189. Excitation and emission maxima of the three insertion mutants were similar to those of wild type, but quantum yields of mutants OMEGA172 and OMEGA189 were lower, indicating increased freedom of the fluorophore. Trypsin cleaved the native (folded) form of each mutant at a unique site defined by the insert. Pronase also yields similar digestion patterns in these variants, but further proteolysis was also observed, suggesting that the primary cleavage relaxes **GFP** structure and reveals previously inaccessible sites. Fluorescence of OMEGA189 changed little upon digestion with trypsin but decreased progressively by as much as 40% upon digestion with increasing amounts of pronase. Fluorescence of other variants was not affected significantly by the proteases, further confirming the remarkable stabilities of **GFP** variants. These constructs define a new conformation-sensitive site around residue 189 of **GFP** and show that **GFP** may be useful for design of protease-susceptible molecules for monitoring of specific proteolytic activities in vivo.
CC Biochemistry studies - General 10060
Biochemistry studies - Proteins, peptides and amino acids 10064
Enzymes - General and comparative studies: coenzymes 10802
Physiology and biochemistry of bacteria 31000
IT Major Concepts
Biochemistry and Molecular Biophysics
IT Chemicals & Biochemicals
factor Xa; green fluorescent protein; pronase; protease; trypsin
IT Methods & Equipment
mutagenesis: analytical method, genetic engineering, genetic method
IT Miscellaneous Descriptors
protease-sensitive insertions; proteolysis
ORGN Classifier
Bacteria 05000
Super Taxa
Microorganisms
Organism Name
bacteria
Taxa Notes
Bacteria, Eubacteria, Microorganisms
RN 9002-05-5 (factor Xa)
9036-06-0 (pronase)
9001-92-7 (protease)
9002-07-7 (trypsin)

ANSWER 1 OF 1 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
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TI Green fluorescent protein rendered susceptible to proteolysis: Positions for protease-sensitive insertions.
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CC Biochemistry studies - General 10060
Biochemistry studies - Proteins, peptides and amino acids 10064
Enzymes - General and comparative studies: coenzymes 10802
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IT Major Concepts
Biochemistry and Molecular Biophysics
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factor Xa; green fluorescent protein; pronase; protease; trypsin
IT Methods & Equipment
mutagenesis: analytical method, genetic engineering, genetic method
IT Miscellaneous Descriptors
protease-sensitive insertions; proteolysis
ORGN Classifier
Bacteria 05000
Super Taxa
Microorganisms
Organism Name
bacteria
Taxa Notes
Bacteria, Eubacteria, Microorganisms
RN 9002-05-5 (factor Xa)
9036-06-0 (pronase)
9001-92-7 (protease)
9002-07-7 (trypsin)

ANSWER 3 OF 11 CAPLUS COPYRIGHT 2004 ACS on STN
AN 2003:296076 CAPLUS
DN 138:315791
ED Entered STN: 17 Apr 2003
TI Fusions of random peptide libraries in scaffold proteins such as green fluorescent protein or β-lactamase
IN Anderson, David; Peelle, Beau Robert; Bogenberger, Jakob Maria
PA Rigel Pharmaceuticals, Inc., USA
SO U.S., 63 pp., Cont.-in-part of U.S. 6,180,343.
CODEN: USXXAM
DT Patent
LA English
IC ICM C07K004-00
ICS C07K014-435; C12Q001-68; C12N015-63; C12N015-12
NCL 530300000; 530350000; 435006000; 435320100; 536023400; 536023500
CC 3-1 (Biochemical Genetics)
Section cross-reference(s): 9
FAN.CNT 4

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	US 6548632	B1	20030415	US 1999-415765	19991008
	US 6180343	B1	20010130	US 1998-169015	19981008
	US 6548249	B1	20030415	US 2000-626581	20000727
	US 6562617	B1	20030513	US 2000-626580	20000727
	US 2001003650	A1	20010614	US 2000-749959	20001227
	US 6596485	B2	20030722		
	US 2003143562	A1	20030731	US 2002-177725	20020620
	US 2003224412	A1	20031204	US 2003-393449	20030318
PRAI	US 1998-169015	A2	19981008		
	US 1999-415765	A3	19991008		
	US 2002-177725	A2	20020620		

AB The invention relates to the use of scaffold proteins, particularly green fluorescent protein (**GFP**) and β-lactamase TEM-1, in fusion constructs with random and defined peptides and peptide libraries. The fusions act to increase the cellular expression levels, decrease the cellular catabolism, increase the conformational stability relative to linear peptides, and increase the steady state concns. of the random peptides and random peptide library members expressed in cells for the purpose of detecting the presence of the peptides and screening random peptide libraries. N-terminal, C-terminal, dual N- and C-terminal, and one or more internal fusions are all contemplated. Internal fusions in Renilla **GFP** may be made in loops 1-5 (amino acid residues 130-135, 154-159, **172**-175, 188-193, or 208-216) for optimal presentation of the peptide. Inclusion of multiple highly flexible amino acid residues between **GFP** and the library allows minimal conformational constraints on the **GFP**. Designed insertion sites in loops 2-4 retain a high level of **GFP** fluorescence when the inserts are flanked by multiple glycines in the tetrapeptide linkers. Novel fusions utilizing self-binding peptides to create a conformationally stabilized fusion domain are also contemplated.
ST scaffold protein fusion random peptide library; green fluorescent protein fusion random peptide library; lactamase fusion random peptide library
IT Animal cell line
(293, **GFP** fusions expressed in; fusions of random peptide libraries in scaffold proteins such as green fluorescent protein or β-lactamase)
IT Aequorea
Renilla reniformis
(**GFP** from; fusions of random peptide libraries in scaffold proteins such as green fluorescent protein or β-lactamase)
IT Animal cell line
(JURKAT, **GFP** fusions expressed in; fusions of random peptide

libraries in scaffold proteins such as green fluorescent protein or β-lactamase)

IT Antigen presentation
Peptide library
(fusions of random peptide libraries in scaffold proteins such as green

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     (FILE 'HOME' ENTERED AT 15:24:06 ON 26 APR 2004)

     FILE 'BIOSIS, CAPLUS, EMBASE, MEDLINE, CANCERLIT, JAPIO' ENTERED AT
     15:24:34 ON 26 APR 2004
L1            19 S GFP AND 157
L2            24 S GFP AND 172
L3             4 S L1 AND L2
L4             1 DUPLICATE REMOVE L3 (3 DUPLICATES REMOVED)
L5             6 DUPLICATE REMOVE L1 (13 DUPLICATES REMOVED)
L6            11 DUPLICATE REMOVE L2 (13 DUPLICATES REMOVED)

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d his

     (FILE 'HOME' ENTERED AT 15:24:06 ON 26 APR 2004)

     FILE 'BIOSIS, CAPLUS, EMBASE, MEDLINE, CANCERLIT, JAPIO' ENTERED AT
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L1            19 S GFP AND 157
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L5             6 DUPLICATE REMOVE L1 (13 DUPLICATES REMOVED)
L6            11 DUPLICATE REMOVE L2 (13 DUPLICATES REMOVED)

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